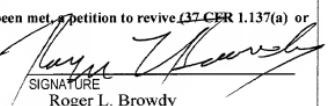


U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE		ATTORNEY'S DOCKET NUMBER KIMCHI 28 09/719748
TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371		U.S. APPLICATION NO. (If known, see 37 CFR 1.5)
INTERNATIONAL APPLICATION NO. PCT/US99/13411	INTERNATIONAL FILING DATE 15 June 1999	PRIORITY CLAIMED 15 June 1998
TITLE OF INVENTION DAP-KINASE RELATED PROTEIN <div style="text-align: center; margin-top: 10px;">  DEC 15 2000 U.S. PATENT & TRADEMARK OFFICE </div>		
APPLICANT(S) FOR DO/EO/US Adi KIMCHI		
<p>Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:</p> <p>1. <input checked="" type="checkbox"/> This is a FIRST submission of items concerning a filing under 35 U.S.C. 371. 2. <input type="checkbox"/> This is a SECOND or SUBSEQUENT submission of items concerning a filing under 35 U.S.C. 371. 3. <input checked="" type="checkbox"/> This is an express request to begin national examination procedures (35 U.S.C. 371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and PCT Articles 22 and 39(1). 4. <input checked="" type="checkbox"/> The US has been elected in a Demand by the expiration of 19 months from the priority date (PCT Article 31). 5. <input checked="" type="checkbox"/> A copy of the International Application as filed (35 U.S.C. 371(c)(2)) a. <input type="checkbox"/> is attached hereto (required only if not transmitted by the International Bureau). b. <input checked="" type="checkbox"/> has been communicated by the International Bureau. c. <input type="checkbox"/> is not required, as the application was filed in the United States Receiving Office (RO/US). 6. <input type="checkbox"/> An English language translation of the International Application as filed (35 U.S.C. 371(c)(2)). 7. <input checked="" type="checkbox"/> Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3)) a. <input type="checkbox"/> are transmitted herewith (required only if not transmitted by the International Bureau). b. <input type="checkbox"/> have been communicated by the International Bureau. c. <input type="checkbox"/> have not been made; however, the time limit for making such amendments has NOT expired. d. <input checked="" type="checkbox"/> have not been made and will not be made. 8. <input type="checkbox"/> An English language translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)). 9. <input type="checkbox"/> An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)). 10. <input type="checkbox"/> An English language translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)).</p>		
<p>Items 11. to 16. below concern document(s) or information included:</p> <p>11. <input type="checkbox"/> An Information Disclosure Statement under 37 CFR 1.97 and 1.98. 12. <input type="checkbox"/> An Assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included. 13. <input checked="" type="checkbox"/> A FIRST preliminary amendment. <input checked="" type="checkbox"/> A SECOND or SUBSEQUENT preliminary amendment. 14. <input type="checkbox"/> A substitute specification. 15. <input type="checkbox"/> A change of power of attorney and/or address letter. 16. <input checked="" type="checkbox"/> Other items or information: <input checked="" type="checkbox"/> Courtesy copy of the International Application as filed. <input checked="" type="checkbox"/> Courtesy copy of the first page of the International Publication (WO 99/66030). <input checked="" type="checkbox"/> Courtesy copy of the International Preliminary Examination Report. There were no annexes. <input checked="" type="checkbox"/> Formal drawings, 16 sheets, Figures 1 - 12 B. <input checked="" type="checkbox"/> Courtesy Copy of the International Search Report.</p>		

U.S. APPLICATION NO. (If known, see 37 CFR 1.5)	International Application No.	Attorney's Docket No.		
09/719748	PCT/US99/13411	KIMCHI 2A		
17. [xx] The following fees are submitted:		CALCULATIONS PTO USE ONLY		
BASIC NATIONAL FEE (37 CFR 1.492 (a)(1)-(5):				
Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO and International Search Report not prepared by the EPO or JPO.....		\$1000.00		
International preliminary examination fee (37 CFR 1.482) not paid to USPTO but International Search Report prepared by the EPO or JPO.....		\$860.00		
International preliminary examination fee (37 CFR 1.482) not paid to USPTO but international search fee (37 CFR 1.445(a)(2)) paid to USPTO.....		\$710.00		
International preliminary examination fee paid to USPTO (37 CFR 1.482) but all claims did not satisfy provisions of PCT Article 33(1)-(4).....		\$690.00		
International preliminary examination fee paid to USPTO (37 CFR 1.482) and all claims satisfied provisions of PCT Article 33(1)-(4).....		\$100.00		
ENTER APPROPRIATE BASIC FEE AMOUNT =				
Surcharge of \$130.00 for furnishing the oath or declaration later than [] 20 [X] 30 months from the earliest claimed priority date (37 CFR 1.492(e)).		\$ 1,000.00		
Claims as Originally Presented		Number Filed	Number Extra	Rate
Total Claims		26 - 20	6	X \$18.00 \$ 108.00
Independent Claims		3 - 3	0	X \$80.00 \$
Multiple Dependent Claims (if applicable)				+\$270.00 \$
TOTAL OF ABOVE CALCULATIONS =		\$ 1,238.00		
Claims After Post Filing Prel. Amend		Number Filed	Number Extra	Rate
Total Claims		30 - 26	4	X \$18.00 \$ 72.00
Independent Claims		3 - 3	0	X \$78.00 \$ 0
TOTAL OF ABOVE CALCULATIONS =		\$ 1,310.00		
Reduction of 1/2 of filing by small entity, if applicable. Applicant claims small entity status. See 37 CFR 1.27.		\$		
SUBTOTAL =		\$ 1,310.00		
Processing fee of \$130.00 for furnishing the English translation later than [] 20 [] 30 months from the earliest claimed priority date (37 CFR 1.492(f)).		\$		
TOTAL NATIONAL FEE =		\$ 1,310.00		
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31). \$40.00 per property +		\$		
TOTAL FEES ENCLOSED =		\$ 1,310.00		
		Amount to be:	\$	
		refunded	\$	
		charged	\$	
a. [] A check in the amount of \$ _____ to cover the above fees is enclosed. b. [X] Credit Card Payment Form (PTO-2038), authorizing payment in the amount of \$ 1,310.00, is attached. c. [] Please charge my Deposit Account No. 02-4035 in the amount of \$ _____ to cover the above fees. A duplicate copy of this sheet is enclosed. d. [XX] The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. 02-4035 . A duplicate copy of this sheet is enclosed.				
NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.				
SEND ALL CORRESPONDENCE TO:				
BROWDY AND NEIMARK, P.L.L.C. 624 NINTH STREET, N.W., SUITE 300 WASHINGTON, D.C. 20001 TEL: (202) 628-5197 FAX: (202) 737-3528 Date of this submission: December 15, 2000				
 SIGNATURE Roger L. Browdy NAME 25,618 REGISTRATION NUMBER				

09/719748

JC01 Rec'd PCT/PTO 15 DEC 2000

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:) Art Unit:
Adi KIMCHI)
)
)
)
IA No.: PCT/US99/13411)
) Washington, D.C.
IA Filed: 15 June 1999)
)
U.S. App. No.:)
 (Not Yet Assigned))
) December 15, 2000
National Filing Date:)
 (Not Yet Received))
)
For: DAP-KINASE RELATED PROTEIN) Docket No.: KIMCHI 2A

PRELIMINARY AMENDMENT

Honorable Commissioner for Patents and Trademarks
Washington, D.C. 20231

Sir:

Contemporaneous with the filing of this case and
prior to calculation of the filing fee, kindly amend as
follows:

IN THE SPECIFICATION

After the title please insert the following
paragraph:

The present application is the national stage under
35 U.S.C. 371 of PCT/US99/13411, filed 15 June 1999. --

702/220-34676260

IN THE CLAIMS

Claim 18, lines 1 & 2 , delete "any of claims 2-7, 9-12 and 14-17", and insert therefor --claim 2--.

Claim 19, lines 1 & 2, delete "any of claims 2-7, 9-12, and 14-17", and insert therefor --claim 2--.

Claim 20, lines 1 & 2, delete "any one of claims 1, 8 and 13", and insert therefor --claim 1--.

Claim 21, line 2, delete "any one of claims 1, 8 and 13", and insert therefor --claim 1--.

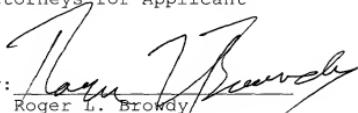
REMARKS

The above amendment to the specification is being made to insert reference to the PCT application of which the present case is a U.S. national stage. The above amendments to the claims are being made in order to eliminate any properly multiply dependent claims, for the purpose of reducing the filing fee. Please enter this amendment prior to calculation of the filing fee in this case.

Favorable consideration is earnestly solicited.

Respectfully submitted,
BROWDY AND NEIMARK, P.L.L.C.
Attorneys for Applicant

By:


Roger L. Browdy
Registration No. 25,618

RLB:wrd
Telephone No.: (202) 628-5197
Facsimile No.: (202) 737-3528

09/719748

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:)	Art Unit:
Adi KIMCHI)	
)	
)	
)	
IA No.: PCT/US99/13411)	
)	Washington, D.C.
IA Filed: 15 June 1999)	
)	
U.S. App. No.:)	
(Not Yet Assigned))	
)	December 15, 2000
National Filing Date:)	
(Not Yet Received))	
)	
For: DAP-KINASE RELATED PROTEIN)	Docket No.: KIMCHI 2A

SUPPLEMENTAL PRELIMINARY AMENDMENT

Honorable Commissioner for Patents and Trademarks
Washington, D.C. 20231

Sir:

Prior to examination upon the merits, kindly amend as follows:

IN THE CLAIMS

Please add the following claims:

--27. A composition comprising a polypeptide according to claim 8, and a pharmaceutically acceptable excipient, carrier, diluent or auxiliary agent.

28. A composition comprising a polypeptide according to claim 13, and a pharmaceutically acceptable excipient, carrier, diluent or auxiliary agent.

29. A molecule containing an antigen binding portion of an antibody which specifically recognizes the polypeptide according to claim 8, with the proviso that said antibody does not cross-react with DAP kinase or ZIP kinase.

30. A molecule containing an antigen binding portion of an antibody which specifically recognizes the polypeptide according to claim 13, with the proviso that said antibody does not cross-react with DAP kinase or ZIP kinase.

REMARKS

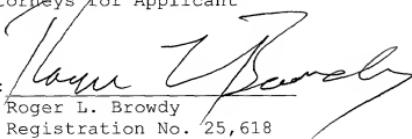
Claims 1-30 presently appear in this case. The above amendments to the claims are being made in order to add new claims and to restore at least partly the varying scope of claims which was eliminated by the elimination of multiple dependencies in the claims.

Favorable consideration is earnestly solicited.

Respectfully submitted,

BROWDY AND NEIMARK, P.L.L.C.
Attorneys for Applicant

By:


Roger L. Browdy
Registration No. 25,618

RLB:wr
Telephone No.: (202) 628-5197
Facsimile No.: (202) 737-3528

DAP-KINASE RELATED PROTEIN

JC01 Rec'd PCT/PTO 15 DEC 2000

CROSS-REFERENCE TO RELATED APPLICATIONS

5 The present application is related to and hereby incorporates by reference the entire contents of each of application serial no. 08/810,712, filed March 3, 1997, and application serial no. 08/631,097, filed April 12, 1996, the latter of which has been published as PCT publication WO 95/10630 on April 20, 1995. The present application also claims priority from 10 provisional application 60/089,294, filed June 15, 1998, the entire contents of which are hereby incorporated by reference.

BACKGROUND OF THE INVENTION

Field of the Invention

The present invention is directed to a DAP-kinase related protein.

Description of the Related Art

One of the factors which determines the proliferation state of cells is the balance between the growth-promoting effects of proto-oncogenes and the growth-constraining effects of tumor-suppressor genes. One mechanism by which tumor-suppressor genes exert their growth-constraining effect is by inducing a cell to undergo a physiological type of death. Such a controlled cell death is evident in a multitude of physiological conditions including metamorphosis, synaptogenesis of neurons, death of lymphocytes during receptor repertoire selection, and controlled homeostasis in the bone marrow and other proliferative tissues, etc. This cell death is regulated by the interaction of 25 the cell with other cells or with cell products, for example through the activity of suitable cytokines.

Growth-inhibiting cytokines have a double effect on the target cell. They can either inhibit the proliferation of the cell and/or give rise to cell death. To date, blockage or activation of expression of known tumor-suppressor genes was shown to counteract or 30 enhance, respectively, cytokines inhibition of cells growth (Kimchi, 1992) but did not have any effect on the death- promoting action of cytokines. For example, the growth inhibitory response to cytokines, such as TGF- β , was markedly reduced by the inactivation of the Rb gene, or the response to IL-6 was enhanced by introducing activated p53 genes (Pietenpol et al., 1990; Levy et al, 1993).

Apoptosis is a genetically controlled cell death process which is important in various developmental stages, as well as for cell maintenance and tissue homeostasis (Jacobson et al., 1997). During the last few years, many of the key players in this process have been identified, including receptors, adaptor proteins, proteases, and other positive and negative regulators (Green et al., 1998; White, 1996). One of the positive mediators of apoptosis, which has been cloned by the present inventors, is DAP-kinase (Deiss et al., 1995). This protein was discovered by a functional approach to gene cloning, based on transfections of mammalian cells with anti-sense cDNA libraries and subsequent isolation of death-protective cDNA fragments (Deiss et al., 1995; Deiss et al., 1991; Kimchi, 1998; Kissil et al., 1998; Levy-Strumpf et al., 1998). The anti-sense cDNA of DAP-kinase protected HeLa cells from interferon-gamma-induced cell death, and this property served as the basis for its selection.

DAP-kinase is a calcium/calmodulin-regulated 160 kDa serine/threonine protein kinase associated with actin microfilaments (Deiss et al., 1995; Cohen et al., 1997). Its structure contains at least two additional domains that might mediate interactions with other proteins: ankyrin repeats, and a typical death domain located at the C-terminal part of the protein (Deiss et al., 1995; Cohen et al., 1997). Overexpression of DAP- kinase in various cell lines results in cell death, and this death-promoting effect of DAP-kinase depends on at least three features: the catalytic activity, presence of the death domain, and the correct intracellular localization (Cohen et al., 1997; Cohen et al., 1999). Several independent lines of evidence proved that DAP-kinase is involved in apoptosis triggered by different external signals, including interferon- γ , TNF- α , activated Fas receptors, and detachment of cells from the extracellular matrix (Deiss et al., 1995; Cohen et al., 1997; Cohen et al., 1999; Inbal et al., 1997). A tumor suppressive function was recently attributed to the DAP-Kinase, coupling the control of apoptosis to metastasis (Inbal et al., 1997).

So far, only a few serine/threonine kinases were implicated in the regulation of programmed cell death, either as death- promoting and death-protecting proteins (Anderson, 1997; Bokoch, 1998). One such candidate is the JNK/SAPK (Basu et al., 1998). In one example, it was shown to mediate apoptosis induced by detachment from extracellular matrix (named anoikis) (Cardone et al., 1997). In this system, the JNK pathway is activated by MEKK-1, whose kinase activity is stimulated by caspase cleavage (Cardone et al., 1997). JNK

may antagonize BCL-2 anti-apoptotic effects by phosphorylation (Park et al., 1997; Maundrell et al., 1997).

Another serine/threonine kinase is RIP, which like DAP-Kinase also possesses the death domain. RIP was shown to positively mediate apoptosis in cell cultures (Stanger et al., 1995). However, *in vivo* studies in RIP-deficient mice demonstrated its ability to exert anti-apoptotic effects by mediating the TNF- α - induced TNF- β activation (Kelliher et al., 1998). Other RIP members, RIP2 and RIP 3 were also recently identified and shown to possess pro-apoptotic effects (McCarthy et al., 1998; Sun et al., 1998; Yu et al., 1999).

Among the negative regulators of apoptosis is the protein kinase AKT. This protein was shown to phosphorylate BAD and thereby to prevent it from complexing and blocking the anti-apoptotic activity of BCL-X_L (Datta et al., 1997; del Peso et al., 1997). AKT was also recently shown to phosphorylate pro-caspase-9, thus blocking its normal processing (Cardone et al., 1998).

Recently, the isolation and characterization of novel kinase members, homologous in their catalytic domains to DAP- kinase, was reported (Kawai et al., 1998; Kogel et al., 1998; Sanjo et al., 1998). One protein, named ZIP-kinase, was found to be 80% identical to DAP-kinase within the kinase domain, yet it lacks the CaM-regulatory domain and the other domains and motifs characteristic of DAP-kinase. Zip-kinase contains a leucine zipper domain at the C-terminus and is localized to the nucleus (Kawai et al., 1998; Kogel et al. 1998). The activation of ZIP kinase occurs by a different mechanism involving homo-dimerization, mediated by its leucine zipper domain. However, unlike DAP-kinase, ZIP-kinase is a nuclear protein, which instead of being regulated by a calmodulin-binding domain, is activated by homo-dimerization of its leucine-zipper motifs (Kogel et al., 1998). Another two less conserved nuclear proteins, DRAK1 and DRAK2, which are closely related to each other, and which share 50% identity with the kinase domain of DAP-kinase, were also recently characterized. Like ZIP-kinase, the DRAK1 and DRAK2 proteins also lack the CaM-regulatory domain. The overexpression of these two proteins in NIH3T3 cells induces some morphological changes associated with apoptosis, dependent on the functionality of their kinase domain (Sanjo et al., 1998). Together these kinases form a novel subfamily of serine/threonine kinases, as is evident from multiple sequence and phylogenetic analysis (Inbal et al., 1999).

10/22/2014 8th/26/2011

15

20

25

30

Ectopic expression of the three wild type kinases, but not their catalytically inactive mutants, induced morphological changes characteristic of apoptosis (Kawai et al., 1998; Sanjo et al., 1998). Yet, in the case of ZIP-Kinase, these results are still controversial (Kogel et al., 1998).

Citation of any document herein is not intended as an admission that such document is pertinent prior art, or considered material to the patentability of any claim of the present application. Any statement as to the content or a date of any document is based on information available to the applicant at the time of filing and does not constitute an admission as to the correctness of such a statement.

SUMMARY OF THE INVENTION

A new protein, DAP-Kinase-related 1 protein (DRP-1), which is a novel homologue of DAP-kinase, has been isolated. This novel calmodulin-dependent kinase is a 42kDa serine/threonine kinase which shows a high degree of homology to DAP-kinase both in its catalytic domain and its calmodulin-regulatory region. The catalytic domain of DRP-1 is also homologous to recently identified ZIP-kinase and, to a lesser extent, to the catalytic domains of DRAK1/2.

DRP-1 is localized to the cytoplasm as shown by immunostaining and cellular fractionation assays. *In vitro* kinase assays indicate that wild type DRP-1, but not a kinase inactive mutant, undergoes autophosphorylation and phosphorylates an external substrate in a Ca^{2+}/CaM -dependent manner. Ectopically expressed DRP-1 is able to induce apoptosis in various types of cells; with this killing being dependent on its kinase activity. The dominant negative form of DAP-Kinase (DAPk DD) is a potent blocker of apoptosis induced DRP-1. Thus, DRP-1 may be a death-promoting protein functioning in the biochemical pathway which involves DAP (death-associated protein)-kinase (e.g., forming a cascade of sequential kinases, one directly activating the other). Alternatively, the two kinases may operate to promote cell death in parallel pathways.

The present invention provides for a DRP-1 protein and functional homologues thereof having at least 85% sequence identity to the DRP-1 sequence of SEQ ID NO:2. Also provided is a fragment of DRP-1, which either is capable of inducing cell death or lacks such capability but instead is capable of inhibiting the activity of DRP-1 or a functional homologue

thereof to induce cell death, and a homologous fragment which has at least 85% sequence identity thereto and which has the same properties.

The present invention further provides an isolated DNA molecule encoding for such DRP-1 protein, functional homologues thereof, or fragments thereof. Also included within the scope of the present invention are isolated DNA molecules which hybridize to the nucleotide sequence encoding DRP-1 protein under moderately or highly stringent conditions and encode a calmodulin-dependent serine/threonine kinase having the property of being capable of inducing cell death.

Other further aspects of the present invention include a composition comprising the DRP-1 protein, functional homologues and fragments thereof, and an antibody which specifically recognizes DRP-1 but does not cross-react with DAP kinase or ZIP kinase.

Yet another aspect of the present invention is directed to a single stranded RNA molecule complementary to at least a portion of the mRNA encoding the DRP-1 protein of SEQ ID NO:2. This single stranded antisense RNA molecule can be used in a method of neutralizing DRP-1 mRNA by hybridizing to the DRP-1 mRNA to prevent its translation into DRP-1 protein.

The present invention also provides a method for screening individuals for predisposition to cancer.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows the nucleotide (SEQ ID NO:1) and amino acid (SEQ ID NO:2) sequence of the DAP-kinase homologue, DRP-1. The initiation (ATG) and stop (TAA) codons are boxed. The polyadenylation signal (ATTAAA) is underlined. The kinase domain and the calmodulin regulatory regions are in bold or underlined by a dash, respectively.

Figures 2A-2B show the multiple sequence alignment of the serine/threonine kinase domains (Fig. 2A) of the DAP-kinase-related proteins, DAP-kinase (SEQ ID NO:3), ZIP-kinase (SEQ ID NO:4), DRP-1 (corresponding to residues 13-275 of SEQ ID NO:2), DRAK1 (SEQ ID NO:5) and DRAK 2 (SEQ ID NO:6), conducted according to Hanks and Quinn (1991) with identical amino acids boxed and homologous amino acids shown with gray shading, and the multiple sequence alignment of the calmodulin regulatory regions (Fig. 2B) of DAP-kinase (SEQ ID NO:7), DRP-1 (corresponding to residues 292 to 320 of SEQ ID NO:2), smMLCK (SEQ ID NO:8), CaMKIIa (SEQ ID NO:9), CaMKI (SEQ ID NO:10),

CaMKIV (SEQ ID NO:11), and ZIP-Kinase (SEQ ID NO:12) conducted manually, keeping the conserved (boxed) regions aligned to each other. The corresponding region of ZIP-Kinase which does not contain homology to DAP-Kinase and ZIP-Kinase CAM-regulatory regions is given at the bottom of Fig. 2B.

5 Figure 3A shows Northern blot analysis of polyA+RNA extracted from various cell lines for mRNA expression of DRP-1, Figure 3B show Western blot analysis of *in vitro* transcription and translation of DRP-1, and Figure 3C shows protein expression of DRP-1 in HeLa cells on an immunoblot.

10 Figures 4A and 4B show control COS-7 cells and cellular localization of DRP-1 in COS-7 cells, respectively, and Figure 4C shows a Western blot of fractions from a detergent extraction of COS-7 cells transfected with a pCDNA3 vector expressing either

15 FLAG-tagged DRP-1 or DAP-Kinase.

Figure 5A shows *in vitro* kinase activity of DRP-1 and Figure 5B shows a Western blot of DRP-1 proteins.

20 Figures 6A-6B show fluorescent microscope images of 293 cells transfected by pCDNA3-luciferase as a negative control (Fig. 6A), by pCDNA3-ΔCaM DAP-Kinase as positive control (Fig. 6B), by pCDNA -DRP-1 (Fig. 6C), and by pCDNA3-K42A DRP-1 (Fig. 6D). Apoptotic cells are indicated by arrows.

25 Figure 7 shows the scores of apoptotic cells in a graph of the percentage of apoptotic cells resulting from the transfections of Figs. 6A-6D.

Figures 8A and 8B show DRP-1 protein expression in 293 transfected cells in immunoblots to anti-FLAG antibodies (Fig. 8A) and anti-vinculin antibodies (Fig. 8B).

25 Figure 9A shows that DAP kinase death domain protects from DRP-1 induced apoptosis, and Figure 9B shows an immunoblot of DRP-1 protein expression in 293 transfected cells.

30 Figure 10A shows a schematic representation of a series of generated deletion mutant, and Figure 10B shows an immunoblot containing extracts of 293 cells transiently transfected with GFP and the series of deletion mutants, (DRP-1 fragments, cloned in pCDNA3, and tagged with HA epitope at the C-terminus), as in Figs. 8A and 8B are probed with anti-HA antibodies for DRP-1 detection and anti-vinculin antibodies to quantitate the loaded protein amounts. In Figure 10B, pCDNA3²-luciferase is the negative control.

Figure 11A shows fluorescent microscope images of the transiently transfected cells of Fig. 10B, and Figure 11B shows a graph of the score in percent apoptotic cells in Fig. 11A resulting from co-transfections of 293 cells with 1-2 μ g HA-tagged wild type DRP-1 or various deletion mutants of DRP-1 after 24 hours (average S.D. calculated from triplicates of 5 100 cells each).

Figures 12A and 12B show by Western analysis that the C-terminal part of DRP-1 is required for its homo-dimerization. In Figure 12A, wild type DRP-1 is shown to undergo specific homo-dimerization. The lanes correspond to the following co-transfections (5 μ g of DRP-1 constructs and 20 μ g of RFX1- Δ SmaI constructs/9mm plate): (1) DRP-1-
5 FLAG+RFX1- Δ SmaI-HA (control to rule-out nonspecific attachment of DRP-1 to HA beads or to an irrelevant gene). (2) RFX- Δ SmaI-FLAG+DRP-1-HA (control to rule out nonspecific attachment of DRP-1 to FLAG beads or to an irrelevant gene). (3) DRP-1-FLAG+DRP-1-HA. Both IP directions and their Western blottings are shown. In Figure 12B, truncation of C-terminal 40 amino acids of DRP-1 is shown to abolish its homo-dimerization. The lanes correspond to the following co-transfections (5 μ g of each construct/90mm plate): (1) DRP-1-FLAG+DRP-1-HA (2) DRP-1-FLAG+DRP-1- Δ 40-HA (3) DRP-1-FLAG+DRP-1- Δ 73-HA (4) DRP-1-FLAG+DRP-1- Δ 85-HA. The lower panel quantitate the immunoprecipitation efficiency of DRP-1-FLAG by the anti-FLAG antibodies.

20 **DETAILED DESCRIPTION OF THE INVENTION**

The present invention is based on the discovery by the present inventor of a novel serine/threonine kinase with remarkable homology to the catalytic and CaM-regulatory domains of DAP-kinase. This kinase, named DAP-kinase-related protein 1 (DRP-1), is a 42kDa cytoplasmic protein which exhibits minor associations with insoluble matrix elements. The nucleotide (SEQ ID NO:1) and amino acid (SEQ ID NO:2) sequences of this DRP-1 protein are shown in Fig 1. It is composed of 1742 nucleotides. The predicted initiation and stop codons are boxed, and the polyadenylation signal is underlined. The protein kinase domain is shown in bold letters and corresponds to amino acid residues 13 to 275 of SEQ ID NO:2. This protein displays 80% identity with the catalytic domain of DAP- kinase.
25 The calmodulin-regulatory region is underlined with a dashed line; this region displays high homology to the corresponding region in DAP-kinase. The remainder of the C-terminal short part of the protein (the last 40 amino acid residues corresponding to residues 321 to 360 of

SEQ ID NO:2)differs completely from DAP- kinase. Thus, DAP-kinase-related I protein does not carry all of the other motifs and protein modules characteristic of DAP- kinase. The mRNA expression levels transcribed from this gene are low.

Another protein, ZIP-kinase, which by virtue of its sequence homology to the kinase domain of DAP-Kinase, is also a member of the DAP-Kinase-related proteins subfamily, was recently identified (Kawai et al., 1998; Kogel et al., 1998). Unlike DAP-Kinase and DRP-1, ZIP-kinase is a nuclear protein, which instead of being regulated by a calmodulin-binding domain, is activated only by homo-dimerization via its leucine-zipper motifs (Kawai et al., 1998). To this group of kinases, another two less homologous nuclear proteins, DRAK1 and DRAK2, were recently added (Sanjo et al., 1998). Together they form a novel subfamily of serine/threonine kinases, as is evident from multiple sequence and phylogenetic analyses (Inbal et al., 1999). A multiple sequence alignment of the kinase domain of these serine/threonine kinases is shown in Fig. 2A.

10
15
20
25
30
35
40
45
50
55
60
65
70
75
80
85
90
95
100

To check the cellular functions of DRP-1, the laboratory of the present inventor overexpressed wild type DRP-1 in various cell lines and found that it induced apoptosis as measured by a few parameters. Unlike the wild type DRP-1, a kinase inactive mutant of DRP-1 (DRP-1 K42A), did not induce apoptosis, although it was expressed at a similar level in the transfected cells. *In vitro* kinase assays confirmed that DRP-1 K42A is indeed unable to phosphorylate MLC or under autophosphorylation. Also, a truncated form of DRP-1 which lacks the CaM-regulatory region, could be shown to induce very high levels of apoptosis, in a similar way to the analogous truncation of the CaM-regulatory region of DAP-Kinase (ΔCaM; Cohen et al., 1997). Such dependence on the catalytic activity for the apoptotic function is apparent also in the other members of DAP-kinase-related proteins (Kawai et al., 1998; Sanjo et al., 1998).

In a deletion mutant study, which is also presented in the Example herein, the existence of a positive element responsible for apoptotic induction which is located at the C-terminal part of DRP-1 is confirmed. This C-terminal tail of DRP-1 is also essential for its dimerization. Thus, self-dimerization is a requirement for the functionality of this kinase in apoptotic assays, although this property can be overridden by a further deletion of the CaM-regulatory region. Like DRP-1, ZIP-kinase-induced cell death is also controlled by its ability to undergo homo-dimerization via the C-terminal leucine zipper domain (Kawai et al., 1998). Three point mutations in the leucine zipper domain abolished the homo-dimerization as well as

the ability of ZIP-kinase to undergo autophosphorylation *in vitro* and significantly reduced its ability to induce cell death of NIH 3T3 cells. It seems reasonable to assume that activation of these kinases is achieved by homo-dimerization followed by trans-phosphorylation events.

5 The high homology in the kinase domains of DAP-kinase and DRP-1, and the finding that they are both localized to the cytoplasm (either in soluble or insoluble forms), imply that they may share the same or closely related substrates. The phosphorylation sites for these kinases on the substrate may be either different or identical. Thus, these kinases may cooperate to induce apoptosis in the same cell type or, alternatively, function independently in different cell types, tissues or organs, or in response to different stimuli or time windows.

Another possibility is that these kinases act sequentially along the same signaling pathway to induce apoptosis.

10 The present invention thus provides for the polypeptide of DRP-1 and for a calmodulin-dependent serine/threonine kinase homologue having the properties of DRP-1, such as the ability to phosphorylate protein in a calcium/calmodulin dependent manner and the ability to induce programmed cell death or apoptosis, and having at least 85% sequence identity to the amino acid sequence SEQ ID NO:2 of DRP-1. Preferably, the calmodulin-dependent serine/threonine kinase homologue has at least 90% sequence identity, and more preferably, at least 95% sequence identity to SEQ ID NO:2.

15 The term "sequence identity" as used herein means that the amino acid sequences are compared by alignment according to Hanks and Quinn (1991) with a refinement of low homology regions using the Clustal-X program. Such an amino acid alignment is shown in Figs. 2A and 2B where the identical amino acid residues are presented in boxes (cutoff=50%) and homologous amino acid residues, determined according to the PAM 250 matrix, are presented by gray shading (cutoff=65%).

20 The Clustal-X program referred to in the previous paragraph is the Windows interface for the ClustalW multiple sequence alignment program (Thompson et al., 1994). The Clustal-X program is available over the internet at <ftp://ftp-igbmc.u-strasbg.fr/pub/clustalx/>. Of course, it should be understood that if this link becomes inactive, those of ordinary skill in the art can find versions of this program at other links using standard internet search

25 techniques without undue experimentation. Unless otherwise specified, the most recent version of any program referred herein, as of the effective filing date of the present application, is the one which is used in order to practice the present invention.

1022201862574680

If the above method for determining "sequence identity" is considered to be nonenabled for any reason, then one may determine sequence identity by the following technique. The sequences are aligned using Version 9 of the Genetic Computing Group's GDAP (global alignment program), using the default (BLOSUM62) matrix (values -4 to +11)

5 with a gap open penalty of -12 (for the first null of a gap) and a gap extension penalty of -4 (per each additional consecutive null in the gap). After alignment, percentage identity is calculated by expressing the number of matches as a percentage of the number of amino acids in the claimed sequence.

In addition to the full length polypeptide of DRP-1 or a functional homologue thereto with at least 85% sequence identity, the present invention also provides for a fragment 10 of the DRP-1 protein of SEQ ID NO:2 which either maintains the ability to induce cell death or lacks this ability but instead is capable of inhibiting the cell killing ability of DRP-1 protein or its functional homologue described above. It was unexpectedly discovered by the present inventor that the 40 amino acid C-terminal tail (residues 321 to 360 of SEQ ID NO:2) is critical to induction of cell death. As the action of DRP-1 is dependent on dimerization, the 40 amino acid tail, by itself, can inhibit the ability of DRP-1 to induce cell death by interfering with and preventing DRP-1 from dimerizing. Furthermore, it was also unexpectedly discovered that the catalytic domain, by itself (without the calmodulin regulatory domain and the 40 amino acid C-terminal tail, e.g., amino acid residues 13 to 275 of SEQ ID NO:2), is 20 super-killing. One of ordinary skill in the art can readily obtain fragments of the full length sequence of the present invention using N-terminal amino peptidases or C-terminal carboxypeptidases. Each fragment can then be readily tested to see if it possesses one of the two functions described herein for such fragments, without undue experimentation.

Besides fragments of DRP-1 having the above-mentioned properties, fragments 25 having an amino acid sequence with at least 85% sequence identity to the above fragments of DRP-1, preferably with at least 90% sequence identity, and more preferably with at least 95% sequence identity, and maintaining the cell death induction or inhibition properties of the original fragment, are also comprehended by the present invention.

Also comprehended by the present invention are chemical derivatives of the 30 DRP-1 and functional homologues and fragments thereof, as defined above, where a "chemical derivative" contains additional chemical moieties not normally part of the DRP-1 amino acid sequence. Covalent modifications of the amino acid sequence are included within

15
10
5
F02/220-134/672/600
15

the scope of this invention. Such modifications may be introduced into DRP-1 or fragments thereof by reacting targeted amino acid residues of the peptide with an organic derivatizing agent that is capable of reacting with selected side chains or terminal residues.

Cysteinyl residues most commonly are reacted with alpha-haloacetates (and 5 corresponding amines), such as chloroacetic acid or chloroacetamide, to give carboxymethyl or carboxyamidomethyl derivatives. Cysteinyl residues also are derivatized by reaction with bromotrifluoroacetone, alpha-bromo- beta-(5-imidazoyl)propionic acid, chloroacetyl phosphate, N-alkylmaleimides, 3-nitro-2-pyridyl disulfide, methyl-2-pyridyl disulfide, p-chloromercuribenzoate, 2-chloromercuri-4-nitrophenol, or chloro-7-nitrobenzo-10 2-oxa-1,3-diazole.

Histidyl residues are derivatized by reaction with diethylprocarbonate at pH 5.5-7.0 because this agent is relatively specific for the histidyl side chain. Parabromophenacyl bromide also is useful; the reaction is preferably performed in 0.1 M sodium cacodylate at pH 6.0.

15 Lysinyl and amino terminal residues are reacted with succinic or other carboxylic acid anhydrides. Derivatization with these agents has the effect of reversing the charge of the lysinyl residues. Other suitable reagents for derivatizing alpha-amino acid-containing residues include imidoesters, such as methyl picolinimidate, pyridoxal phosphate, pyridoxal, chloroborohydride, trinitrobenzenesulfonic acid, O-methyliosurea, 20 2,4-pentanedione, and transaminase-catalyzed reaction with glyoxylate.

Arginyl residues are modified by reaction with one or several conventional reagents, among them phenylglyoxal, 2,3-butanedione, and ninhydrin. Derivatization of arginine residues requires that the reaction be performed in alkaline conditions because of the high pKa of the guanidine functional group. Furthermore, these reagents may react with the 25 groups of lysine, as well as the arginine epsilon-amino group.

The specific modification of tyrosyl residues *per se* has been studied extensively, with particular interest in introducing spectral labels into tyrosyl residues by reaction with aromatic diazonium compounds or tetrinitromethane. Most commonly, N-acetylimidazole and tetrinitromethane are used to form O-acetyl tyrosyl species and e-nitro 30 derivatives, respectively.

Carboxyl side groups (aspartyl or glutamyl) are selectively modified by reaction with carbodiimides (R'N-C-N-R') such as 1-cyclohexyl-3-[2-morpholinyl-(4-ethyl)]

carbodiimide or 1-ethyl-3-(4-azonia-4,4-dimethylpenty)carbodiimide. Furthermore, aspartyl and glutamyl residues are converted to asparaginyl and glutaminyl residues by reaction with ammonium ions.

Glutaminyl and asparaginyl residues are frequently deamidated to the 5 corresponding glutamyl and aspartyl residues. Alternatively, these residues are deamidated under mildly acidic conditions. Either form of these residues falls within the scope of this invention.

10 15 20 25 30 35 40 45 50 55 60 65 70 75 80 85 90 95 100

The present invention also comprehends an isolated DNA molecule which includes a nucleotide sequence encoding the DRP-1 protein of SEQ ID NO:2, a functional homologue thereof as described above, or a fragment of DRP-1 which either maintains the ability of DRP-1 to induce cell death or lacks this ability but is instead capable of inhibiting the cell killing ability of DRP-1 protein, as defined above. The isolated DNA molecule according to the present invention is also intended to comprehend a DNA molecule which hybridizes under moderately stringent, preferably highly stringent, conditions to the nucleotide sequence encoding DRP-1 (corresponding to nucleotides 62 to 1141 of SEQ ID NO:1) and which encodes a polypeptide which maintains the cell death induction properties of DRP-1. The present invention further comprehends isolated DNA molecules which hybridize under moderately stringent, preferably highly stringent, conditions to a nucleotide sequence which encodes for a fragment of DRP-1 which either maintains the ability of DRP-1 to induce cell 20 death (i.e., nucleotides 98 to 886 of SEQ ID NO:1 encoding the catalytic kinase domain of DRP-1) or lacks the ability but is instead capable of inhibiting the cell killing ability of DRP-1 protein (i.e., nucleotides 1022 to 1141 of SEQ ID NO:1 encoding the 40 amino acid C-terminal tail of DRP-1). Furthermore, polypeptides encoded by any nucleic acid, such as DNA or RNA, which hybridizes to the nucleotide sequence of nucleotides 62 to 141 of SEQ ID 25 NO:1 under moderately stringent or highly stringent conditions are considered to be within the scope of the present invention as long as the encoded polypeptide maintains the ability of DRP-1 to induce cell death.

As used herein, stringency conditions are a function of the temperature used in the hybridization experiment, the molarity of the monovalent cations and the percentage of 30 formamide in the hybridization solution. To determine the degree of stringency involved with any given set of conditions, one first uses the equation of Meinkoth et al. (1984) for

determining the stability of hybrids of 100% identity expressed as melting temperature Tm of the DNA-DNA hybrid:

$$Tm = 81.5^{\circ}\text{C} + 16.6 (\text{Log}M) + 0.41 (\% \text{GC}) - 0.61 (\% \text{ form}) - 500/L$$

where M is the molarity of monovalent cations, %GC is the percentage of G and C nucleotides in the DNA, % form is the percentage of formamide in the hybridization solution, and L is the length of the hybrid in base pairs. For each 1°C that the Tm is reduced from that calculated for a 100% identity hybrid, the amount of mismatch permitted is increased by about 1%.

Thus, if the Tm used for any given hybridization experiment at the specified salt and formamide concentrations is 10°C below the Tm calculated for a 100% hybrid according to the equation of Meinkoth, hybridization will occur even if there is up to about 10% mismatch.

As used herein, "highly stringent conditions" are those which provide a Tm which is not more than 10°C below the Tm that would exist for a perfect duplex with the target sequence, either as calculated by the above formula or as actually measured.

"Moderately stringent conditions" are those which provide a Tm which is not more than 20°C below the Tm that would exist for a perfect duplex with the target sequence, either as calculated by the above formula or as actually measured. Without limitation, examples of highly stringent ($5\text{--}10^{\circ}\text{C}$ below the calculated or measured Tm of the hybrid) and moderately stringent ($15\text{--}20^{\circ}\text{C}$ below the calculated or measured Tm of the hybrid) conditions use a wash solution of 2 X SSC (standard saline citrate) and 0.5% SDS (sodium dodecyl sulfate) at the appropriate temperature below the calculated Tm of the hybrid. The ultimate stringency of the conditions is primarily due to the washing conditions, particularly if the hybridization conditions used are those which allow less stable hybrids to form along with stable hybrids. The wash conditions at higher stringency then remove the less stable hybrids. A common hybridization condition that can be used with the highly stringent to moderately stringent wash conditions described above is hybridization in a solution of 6 X SSC (or 6 X SSPE (standard saline-phosphate-EDTA)), 5 X Denhardt's reagent, 0.5% SDS, 100 $\mu\text{g}/\text{ml}$ denatured, fragmented salmon sperm DNA at a temperature approximately 20° to 25°C below the Tm. If mixed probes are used, it is preferable to use tetramethyl ammonium chloride (TMAC) instead of SSC (Ausubel, 1987, 19989).

Additional aspects of the present invention are vectors which carry the isolated DNA molecule according to the present invention and a host cell which is transformed with the isolated DNA according to the present invention.

The present invention further provides for antisense RNA complementary to at least a portion of a messenger RNA (mRNA or "sense" RNA) molecule which is the transcription product of the DNA sequence encoding the DRP-1 protein of SEQ ID NO:2. The antisense DRP-1 sequence can be chemically synthesized or it can be expressed in host cells. However, when expressed in host cells, the expressed antisense RNA must be stable (i.e., does not undergo rapid degradation). Moreover, the antisense DRP-1 RNA, will essentially specifically only hybridize to the sense DRP-1 mRNA and form a stable double-stranded RNA molecule that is essentially non-translatable. In other words, the antisense DRP-1 RNA prevents the expressed sense DRP-1 mRNA from being translated into active DRP-1 protein. When expressed in host cells, a vector-borne antisense DRP-1 sequence may carry either the entire DRP-1 gene sequence or merely a portion thereof as long as the antisense DRP-1 sequence is capable of hybridizing to "sense" DRP-1 mRNA to prevent its translation into DRP-1 protein. Accordingly, an "antisense" sequence of the present invention can be defined as a sequence which is capable of specifically hybridizing to "sense" DRP-1 mRNA to form a non-translatable double-stranded RNA molecule.

The antisense DRP-1 sequence need not hybridize to the entire length of the DRP-1 mRNA. Instead, it may hybridize to selected regions, such as the 5'-untranslated sequence, the coding sequence, or the 3'-untranslated sequence of the "sense" mRNA. In view of the size of the mammalian genome, the antisense DRP-1 sequence is preferably at least 17, more preferably at least 30, base pairs in length. However, shorter sequences may still be useful, i.e., they either fortuitously do not hybridize to other mammalian sequences, or such "cross-hybridization" does not interfere with the metabolism of the cell in a manner and to a degree which prevents the accomplishment of an object of this invention. The greater the length of the antisense sequence and the greater the number of complementary base pairs, the greater the number of non-complementary bases that can be tolerated, especially if the non-complementary bases are scattered. Both the preferred hybridization target on DRP-1 and the preferred antisense sequence length are readily determined by systematic experiment.

Standard methods such as described in Sambrooke et al., (1989) can be used to systematically remove an increasingly larger portion of the antisense DRP-1 sequence from a plasmid vector. Besides the full length antisense DRP-1 sequence, a series of staggered deletions may be generated, preferably at the 5'-end of the antisense DRP-1 sequence. This creates a set of truncated antisense DRP-1 sequences that still remain complementary to

15
20
25
30
35
40
45
50
55
60
65
70
75
80
85
90
95

preferably the 5'-end of the sense DRP-1 mRNA and as a result, still forms a RNA molecule that is double-stranded at the 5'-end of the sense DRP-1 mRNA (complements the 3'-end of an antisense DRP-1 RNA) and remains non-translatable.

The antisense RNA according to the present invention can be used in a method

5 to neutralize a mRNA molecule, which is the transcription product of the DNA sequence encoding the DRP-1 protein of SEQ ID NO:2, by allowing the antisense RNA to hybridize to the DRP-1 mRNA to prevent its translation into DRP-1 protein.

A further aspect of the present invention is directed to a composition, such as a pharmaceutical composition, which contains DRP-1, functional homologues or fragments thereof and a pharmaceutically-acceptable excipient, carrier, diluent, or auxiliary agent.

100 An antibody, which specifically recognizes DRP-1 or functional homologues thereof is part of the present invention as long as the antibody does not cross-react with DAP-Kinase or ZIP-kinase. For instance, an antibody that specifically recognizes the unique 40 amino acid C-terminal tail of DRP-1, which is not present in DAP-Kinase or ZIP-kinase, is a preferred embodiment of the antibody according to the present invention. Such an antibody can be used for diagnostic imaging, purification of DRP-1 etc.

105 The terms "antibody" or "antibodies" as used herein are intended to include intact antibodies, such as polyclonal antibodies or monoclonal antibodies (mAbs), as well as proteolytic fragments thereof such as the Fab or F(ab')₂ fragments. Furthermore, the DNA

20 encoding the variable region of the antibody can be inserted into other antibodies to produce chimeric antibodies (see, for example, U.S. Patent 4,816,567) or into T-cell receptors to produce T-cells with the same broad specificity (Eshhar et al., 1990; Gross, et al., 1989). Single chain antibodies can also be produced and used. Single chain antibodies can be single chain composite polypeptides having antigen binding capabilities and comprising a pair of

25 amino acid sequences homologous or analogous to the variable regions of an immunoglobulin light and heavy chain (linked V_H-V_L or single chain F_V). Both V_H and V_L may copy natural monoclonal antibody sequences or one or both of the chains may comprise a CDR-FR construct of the type described in U.S. Patent 5,091,513 (the entire contents of which are hereby incorporated herein by reference). The separate polypeptides analogous to the variable

30 regions of the light and heavy chains are held together by a polypeptide linker. Methods of production of such single chain antibodies, particularly where the DNA encoding the polypeptide structures of the V_H and V_L chains are known, may be accomplished in

accordance with the methods described, for example, in U.S. Patents 4,946,778, 5,091,513 and 5,096,815, the entire contents of each of which are hereby incorporated herein by reference.

As mentioned above, the terms "antibody" or "antibodies" are also meant to

5 include both intact molecules as well as fragments thereof, such as, for example, Fab and F(ab')₂, which are capable of binding antigen. Fab and F(ab')₂ fragments lack the Fc fragment of intact antibody, clear more rapidly from the circulation, and may have less non-specific tissue binding than an intact antibody (Wahl et al., 1983). It will be appreciated that Fab and F(ab')₂ and other fragments of the antibodies useful in the present invention may be used for the detection and quantitation of DRP-1 or functional homologues thereof according to the methods used for intact antibody molecules. Such fragments are typically produced by proteolytic cleavage, using enzymes such as papain (to produce Fab fragments) or pepsin (to produce F(ab')₂ fragments).

10 The present invention comprehends not only the intact antibodies or fragments, but also any molecule which includes an antigen binding portion of an antibody such that the molecule is capable of binding to the antigen. It is well within the skill of the art for the artisan to make e.g., fusion proteins which include antigen binding portions of an antibody fused to any other material which is desired to be carried to the antigen binding site, such as marker molecules, toxins, etc.

20 The antibodies, or fragments of antibodies, of the present invention may be used to quantitatively or qualitatively detect the presence of DRP-1 or functional homologues according to the present invention in a sample. The antibody according to the present invention may also be used for the isolation and purification of DRP-1 or homologues and fragments thereof, such as in an affinity column where the antibodies are immobilized on a

25 solid phase support or carrier.

30 By "solid phase support or carrier" is intended any support capable of binding antigen or antibodies. Well-known supports, or carriers, include glass, polystyrene, polypropylene, polyethylene, dextran, nylon, amyloses, natural and modified celluloses, polyacrylamides, gabbros, and magnetite. The nature of the carrier can be either soluble to some extent or insoluble for the purposes of the present invention. The support material may have virtually any possible structural configuration so long as the coupled molecule is capable of binding to an antigen or antibody. Thus, the support configuration may be spherical, as in a

bead, or cylindrical, as in the inside surface of a test tube, or the external surface of a rod. Alternatively, the surface may be flat such as a sheet, test strip, etc. Those skilled in the art will know many other suitable carriers for binding antibody or antigen, or will be able to ascertain the same by use of routine experimentation.

5 One of the ways in which the DRP-1-specific antibody can be detectably labeled is by linking the same to an enzyme and used in an enzyme immunoassay (EIA). This enzyme, in turn, when later exposed to an appropriate substrate, will react with the substrate in such a manner as to produce a chemical moiety which can be detected, for example, by spectrophotometric, fluorimetric or by visual means. The detection can be accomplished by 10 colorimetric methods which employ a chromogenic substrate for the enzyme. Detection may also be accomplished by visual comparison of the extent of enzymatic reaction of a substrate in comparison with similarly prepared standards.

15 Detection may also be accomplished using any of a variety of other immunoassays. For example, by radioactively labeling the antibodies or antibody fragments, it is possible to detect DRP-1 protein through the use of a radioimmunoassay (RIA) (Chard, T., "An Introduction to Radioimmune Assay and Related Techniques" (In: Work, T.S., *et al.*, Laboratory Techniques in Biochemistry in Molecular Biology, North Holland Publishing Company, New York (1978), incorporated by reference herein). The radioactive isotope can be detected by such means as the use of a gamma counter or a liquid scintillation counter or by 20 autoradiography. Radioactively labeled antibodies or antibody fragments can also be used for their capacity to kill cells bound by such antibodies, or cells in the immediate vicinity which are exposed to the radiation from such antibodies. It is also possible to label the antibody with a fluorescent compound, a chemiluminescent or bioluminescent compound.

25 The antibody molecules of the present invention may also be adapted for utilization in an immunometric assay (also known as a "two-site" or "sandwich" assay) which is well known in the art.

30 In the present specification, the term "programmed cell death" is used to denote a physiological type of cell death which results from activation of some cellular mechanisms, i.e., death which is controlled by the cell's machinery. Programmed cell death may, for example, be the result of activation of the cell machinery by an external trigger, e.g., a cytokine, which leads to cell death. The term "apoptosis" is also used interchangeably with programmed cell death.

The term "tumor" in the present specification denotes an uncontrolled growing mass of abnormal cells. This term includes both primary tumors, which may be benign or malignant, as well as secondary tumors, or metastases, which have spread to other sites in the body.

5 DRP-1 can be used to inhibit growth and metastasis of tumors. Tumor cells are exposed to a variety of death-inducing signals which, in combination with DAP-kinase-related I, can lead to death of the tumor cells. For example, in the blood stream, invading tumor cells must resist programmed cell death that is induced by interactions with cytotoxic T lymphocytes, natural killer cells, and macrophages, and with the cytokines which 10 these hematopoietic cells secrete (e.g., IFNs, TNF, IL-1 β). Tumor cells must also resist the apoptotic cell death induced by nitric oxide anions produced by the endothelial cells, and 15 withstand mechanical shearing forces caused by hemodynamic turbulence. Moreover, during the intravasation or extravasation processes, and during growth in a foreign hostile microenvironment, locally produced inhibitory cytokines (e.g., TGF- β or loss of cell-matrix 20 interactions (e.g., detachment from the basement membranes) also trigger apoptotic cell death.

DRP-1 is useful in promoting death of tumor cells. The protein may be administered to patients, in particular, to cancer patients, which administration may cause death of the tumor cells. The protein may be administered *per se*, or may be administered by an expression vector comprising a DNA molecule of the present invention.

20 Because DRP-1 displays 80% identity with the catalytic domain of DAP-kinase and has a region which displays a high homology to the calmodulin-regulatory region of DAP-kinase, it is expected that DRP-1 has enzymatic kinase activity, which is calmodulin-dependent. Thus, DRP-1 has use as an enzyme and may be used, for example, as the enzyme in any *in vitro* enzymatic reaction which requires the presence of a kinase enzyme.

25 Accordingly, DRP-1 can be used *in vitro* to catalyze phosphorylation reactions as a kinase.

DRP-1 is capable of inducing apoptotic cell death when overexpressed in various cell lines. This ectopic cell death is blocked specifically by the death domain of DAP-kinase, suggesting possible crosstalk between these two kinases. Thus, DRP-1 may also be used for promoting the death of normal or tumor cells and for suppressing the metastatic 30 activity of tumor cells. A particular application of the death-promoting aspect is in therapy of diseases or disorders associated with uncontrolled, pathological cell growth, e.g., cancer (primary tumors and metastasis), psoriasis, autoimmune disease and others. Indeed, it is

expected that the DAP-kinase-related protein I of the present invention and DNA encoding it, may be used in the same manner as disclosed in detail in U.S. applications 08/810,712 and 08/631,097, as well as WO 95/10630.

According to a further aspect of the present invention, referred to herein at 5 times as "the screening aspect", DRP-1 DNA molecules are used in order to screen individuals for predisposition to cancer. In accordance with this aspect the screening is carried out by comparing the sequence of each of the DAP-kinase-related I DNA molecules to each of the respective DAP genes in the individual, or by following RNA and/or protein expression. The absence of a DAP-kinase-related I gene, a partial deletion or any other difference in the sequence that indicates a mutation in an essential region, or the lack of a DRP-1 RNA and/or protein which may result in a loss of function may lead to a predisposition for cancer. For screening, preferably a battery of related DAP and DRP-1 genes may be used, as well as different antibodies.

In the screening aspect, DAP-kinase related product I molecules may also be used for prognostic purposes. For example, if a tumor cell lacks DRP-1 activity, this may reflect high chances of developing metastasis. In addition, DRP-1 positive cells may be more susceptible to control by chemotherapeutic drugs that work by inducing apoptosis, so that the choice of treatment modalities may be made based upon the DRP-1 state of the cells.

The DAP-kinase-related product can be used to screen individuals for 20 predisposition to cancer. There is provided a method for detecting the absence of a DRP-1 gene, a partial deletion or a mutation (i.e., point mutation, deletion or any other mutation) in the DRP-1 genes of an individual, or the absence of a DRP-1 RNA or protein, comprising probing genomic DNA, cDNA, or RNA from the individual with a DNA probe or a multitude of DNA probes having a complete or partial sequence of the DRP-1 genes, or probing protein 25 extracts with specific antibodies.

A particular application of the screening aspect of this invention is in the screening for individuals having a predisposition to cancer, an absence of the gene; or a detected mutation or deletion indicating that the individual has such a predisposition.

One example of a method in accordance with the screening aspect typically 30 comprises the following steps:

(a) obtaining a sample of either genomic DNA from cells of the individual or cDNA produced from mRNA of said cells;

(b) adding one or more DNA probes, each of said probes comprising a complete or partial sequence of a DRP-1 gene;

(c) providing conditions for hybridization to determine whether the DRP-1 gene is present or absent, i.e., whether there is a match between the sequence of the DNA probe or probes and a sequence in the DNA of said sample or a mismatch, a mismatch indicating a deletion or a mutation in the endogenous DNA and a predisposition to cancer in the tested individual.

5

Other examples of the screening aspect of the invention are well known to the skilled artisan and include, but are not limited to, Northern blots, RNase protection assays, and 10 various PCR procedures.

15 The mutation in the DRP-1 gene, indicating a possible predisposition to cancer, can also be detected by the aid of appropriate antibodies which are able to distinguish between a mutated, a non-functional and a normal functional DRP-1 gene product. In addition, mutations that abolish protein translation or transcription due to promoter inactivation can be detected with the aid of antibodies that are used to react with protein cell extracts. Screening is also possible with respect to metastases.

20 Having now generally described the invention, the same will be more readily understood through reference to the following example which is provided by way of illustration and is not intended to be limiting of the present invention.

EXAMPLE

25 In this study, the identification and the structure/function analysis of a novel DAP-kinase-related protein, DRP-1, is described, DRP-1 is a 42kDa Ca^{2+} /CaM-regulated serine/threonine kinase which shows high degree of homology to DAP (Death Associated Protein)-kinase. The homology spans over the catalytic domain and the calmodulin-regulatory region, whereas the rest C-terminal part of the protein differs completely from DAP-kinase and displays no homology to any known protein. The catalytic domain is also homologous to the recently identified ZIP-kinase and to a lesser extent to the catalytic domains of DRAK1/2, thus forming together a novel subfamily of serine/threonine kinases. DRP-1 is localized to the 30 cytoplasm as shown by immunostaining and cellular fractionation assays. *In vitro* kinase assays indicate that wild type DRP-1, but not a kinase inactive mutant, undergoes autophosphorylation and phosphorylates an external substrate in a Ca^{2+} /CaM-dependent

10/22/98 4:16:57 PM

manner. Ectopically expressed DRP-1 is able to induce apoptosis in various types of cells. Cell killing by DRP-1 is dependent on two features: the intact kinase activity and the presence of C-terminal 40 amino acids shown to be involved in self-dimerization of the kinase. Interestingly, further deletion of the CaM-regulatory region overrided the indispensable role of the C-terminal tail and generated a "super-killer" mutant. Finally, a dominant negative fragment of DAP-kinase encompassing the death domain is a potent blocker of apoptosis induced by DRP-1. This implies a possible functional connection between DAP-kinase and DRP-1. The experiments conducted in this study and the results obtained are presented below.

10
15
20
25
30

MATERIALS AND METHODS

cDNA cloning and Northern Blot Analysis

A PCR fragment of 364 bp was obtained from a λgt11 human spleen cDNA library (Clontech) using primers from the deduced DRP-1 sequence, 1047-GGCCGGATGAGGACCTGAGG-1066 (SEQ ID NO:13) and 1411- TCCACACTCCCACCCAGACTC-1390 (SEQ ID NO:14). To obtain the full length cDNA of DRP-1, the same cDNA library was screened with the radiolabeled PCR product. Positive phage clones were isolated, cDNA was subcloned into a BlueScript vector and analyzed by restriction enzyme mapping and DNA sequencing. A 270 bp 3'-fragment from the full length cDNA of DRP-1 was generated by EcoRI digestion, and used to probe polyA+ RNA prepared by a standard procedure from various cell lines.

In vitro Transcription and Translation Assay

The full length cDNA was used as a template for *in vitro* transcription from the T7 promoter. This RNA was translated in reticulocyte lysate (TNT® T7 Quick Coupled Transcription/Translation System; Promega) by conventional procedures, with [³⁵S] methionine (Amersham) as a labeled precursor. The reaction product was then run on 12% SDS-PAGE gel, followed by sodium salicylate incubation for signal amplification. The gel was dried and exposed to X-ray film.

In vitro Kinase Assay

293 cells were transfected by a FLAG-tagged wild type DRP-1, DRP-1 K42A mutant, or mock-transfected. Cell lysates of 293 transfected cells were prepared as previously described (Deiss et al., 1995). Immunoprecipitation of DRP-1 or DRP-1 K42A mutant from 150 μ g total extract was done with 20 μ l anti-FLAG M2 gel (IBI, Kodak) in 500 μ l of PLB 5 supplemented with protease and phosphatase inhibitors for 2h at 4°C. Following three washes with PLB, the immunoprecipitates were washed once with reaction buffer (50 mM HEPES pH 7.5, 20 mM MgCl₂, and 0.1 mg/ml BSA). The proteins bound to the beads were incubated for 15 min at 30°C in 50 μ l of reaction buffer containing 15 μ Ci [γ 32p] ATP (3 pmole), 50 μ M ATP, 5 μ g MLC (Sigma), and where indicated, also 1 μ M bovine calmodulin (Sigma), 0.5 mM 10 CaCl₂, or 3mM EGTA in the absence of calmodulin/CaCl₂. Protein sample buffer was added to terminate the reaction, and after boiling, the proteins were analyzed on 11% SDS-PAGE. The gel was blotted onto a nitrocellulose membrane and ³²P- labeled proteins were visualized by autoradiography.

104220-1514677600

15 Immunostaining of Cells

DRP-1 transfected or mock-transfected COS-7 cells were plated on glass cover-slips (13 mm diam.). After 48 hours, the cells were fixed/permeabilized in 3% formaldehyde for 5 min, methanol 5 min, acetone 2 min. The cells were blocked in 10% NGS for 30 min and incubated with anti-FLAG antibodies (dilution 1:100; IBI, Kodak) in 10% 20 NGS for 60 min. Rhodamine-conjugated goat anti-mouse secondary antibodies (dilution 1:200; Jackson Immuno Research Lab.) and the nucleic acid dye, Oligreen (dilution 1:5000; Molecular Probes), for nuclear staining were then applied. The coverslips were mounted in Mowiol and observed under fluorescence microscope.

25 Detergent Extraction Assay

Sub-confluent cultures of COS-7 transfected cells, grown on 9 cm plate, were washed once with PBS and then with MES buffer (50 mM MES pH 6.8, 2.5 mM EGTA, 2.5 mM MgCl₂). The cells were extracted for 3 min with 0.5 ml of 0.5% Triton X-100 in MES buffer supplemented with protease inhibitors. The supernatant (the soluble fraction- Sol) was 30 collected, centrifuged for 2 min. at 16,000x g at 4°C, and the clear supernatant was then transferred to new tubes. Two volumes of cold ethanol were added and the tubes were incubated at -20°C for overnight, centrifuged 10 min. at 16,000x g at 4°C and resuspended in

200 μ l of 2x protein sample buffer without dye. The detergent insoluble matrix (InSol) remaining on the plate was extracted in 200 μ l of 2x protein sample buffer, scraped from the plate with rubber policeman and collected into tube. The samples were loaded on 10% SDS-PAGE, 100 μ g protein extracts were loaded on each lane from the Sol fraction, 5 equivalent volumes of InSol were loaded. Analysis of the proteins was done using monoclonal anti-FLAG antibodies (dilution 1:200; IBI, Kodak).

Cell Lines, Transfections and Apoptotic Assays

10 All cell lines were grown in DMEM (Biological Industries) supplemented with 10% fetal calf serum (Bio-Lab). For transient transfection, 1x10⁵ cells per well, were seeded in a 6 well plate a day before transfection. Transfections were done by calcium-phosphate method. For cell death assays by inducing overexpression, a mixture containing 1.5 μ g of cell death plasmid (expressing either DRP-1 or Δ CaM DAPk mutant) and 0.5 μ g of pEGFP-NI plasmid (Clontech) was used. For cell death protection assays we used a mixture containing 15 1.2 μ g of cell death inducing plasmid (either DRP-1 or Δ CaM DAPk mutant), 0.5 μ g of a plasmid to be tested for cell death protection (expressing DAPk-DD, DN FADD or luciferase as negative control), and 0.5 μ g of pEGFP-NI plasmid. Cells were counted and photographed 24 hours after transfection. In each transfection, three fields, each consisting of at least 100 GFP-positive cells, were scored for apoptotic cells according to their morphology. When 20 indicated, cell lysates were prepared from the transient transfection at 24 hours, for protein analysis. The transfections of Rat embryo fibroblasts (REF) and FACS analysis of transfected fibroblasts for DNA content distribution were done as previously described (Kissil et al., 1998).

25 Co-immunoprecipitation assays

293 cells grown in 90mm plates (1x10⁶ cells/plate) were co-transfected with 5 μ g FLAG-tagged or HA-tagged DRP-1 and 20 μ g of HA-tagged or FLAG-tagged RFX1- Δ SmaI, respectively, or with DRP-1-HA and DRP-1-FLAG, 5 μ g each. Immunoprecipitation of DRP-1 or RFX1- Δ SmaI from 1mg total extract was done using anti-FLAG M2 gel or anti-30 HA as described above. Detection of bound proteins was done using anti-HA antibodies (dilution 1:1000, Babco) or anti-FLAG antibodies. For the deletion mutant study, 5 μ g of FLAG-tagged full length DRP-1 were co-transfected with 5 μ g of HA-tagged DRP-1 deletion

mutants. Immunoprecipitation of DRP-1 from 1mg total extract was done using anti-FLAG M2 gel as described above. Detection of co-immunoprecipitated proteins (the mutants of DRP-1 or full length DRP-1) was done using anti-HA antibodies.

5 Nucleotide sequence accession number

The nucleotide sequence of human DRP-1 has been submitted to the GenBank™/EBI Data Bank (accession no. AF052941). The murine DRP-1 is also deposited at the GenBank™/EBI Data Bank (accession no. AF052942).

10 RESULTS

Cloning of DRP-1

To identify proteins that share homologous sequences with DAP-kinase, EST databases were searched using the BLAST™ program. Two ESTs of human and murine origin showed remarkable amino acid homology to the catalytic domains of DAP-kinase and the recently identified protein ZIP-kinase (79.5% and 80.2% identity, respectively). A second EST search was performed using the 5' and the 3' ends of the human EST, which identified a few more overlapping ESTs. A putative novel cDNA sequence was generated and used to design primers for cloning the full length cDNA. PCR performed on human spleen cDNA library amplified a 364 bp fragment that was further used to screen the same library. The full length cDNA was then isolated, subcloned into BlueScript vector, and sequenced.

The isolated cDNA was found to be 1742 bp long and to contain a serine/threonine kinase domain with all of the 12 characterized subdomains present (Park et al., 1997, Fig. 1A). Sequence alignment indicated that the catalytic domain of DRP-1 has 80% sequence identity to that of DAP-kinase and ZIP-kinase, yet less 50% sequence identity to the newly identified DRAK proteins (Fig. 2A). Like DAP-kinase but unlike ZIP-kinase, DRP-1 carries a typical CaM-regulatory region adjacent to its catalytic domain, as shown in Figures 1 and 2B. As compared with other kinases such as CaKIIa and MLCK, DRP-1 has the highest homology to DAP-kinase in this region, as shown in Figure 2B. The remaining short stretch of amino acids at the C-terminal part of DRP-1 (40 amino acid tail) displays no homology to any known protein.

30 Expression of DRP-1 and Tissue Distribution

To check the RNA expression of DRP-1, polyA+RNA was prepared from various cell lines and hybridized to a probe designed from the less conserved region of DRP-1. A single weak band of 1.9 kb appeared in some cell lines, in a Northern blot analysis of poly A+RNA (3 micrograms) extracted from various cell lines (Fig. 3A), suggesting that the 5 mRNA is expressed at low amounts in HeLa, 293 and MCF-7 cells. The mRNA was hybridized with a radiolabeled human DRP-1 probe. The position of the transcript is indicated by an arrow. From PCR analysis of various cDNA libraries and the data gathered from EST searches, it was concluded that human DRP-1 is expressed, at least, in spleen, colon, breast, and leukocyte tissue.

10 *In vitro* transcription and translation assays conducted in reticulocyte lysates using the cloned DRP-1 cDNA as a template generated a single protein band of about 42 kDa in size, as predicted by its sequence. This protein band, obtained by Western blot analysis of *in vitro* transcribed and then translated DRP-1, is shown in Figure 3B. A FLAG-tagged DRP-1 was then cloned into pCDNA3 vector and expressed in HeLa cells. A protein of 42 kDa was evident upon immunoblot analysis of the cell lysates with anti-FLAG antibodies, shown in Figure 3C. In this case, 24 hours following transfection, the cells were harvested and lysed. The extracted proteins were separated by SDS-PAGE and then immunoblotted with anti-FLAG antibodies.

20 **Cellular Localization of Ectopically Expressed DRP-1**

In order to follow the cellular localization of the exogenous DRP-1, FLAG-tagged DRP-1 was expressed in COS-7 cells. COS-7 cells were transfected by a FLAG-tagged DRP-1 cloned in pCDNA3 vector, fixed and permeabilized in 1% formaldehyde followed by methanol/acetone treatment. Cells were visualized under fluorescence 25 microscope. Immunoblot analysis proved that DRP-1 was expressed in these cells. For the immunostaining procedure, the non-transfected (Fig. 4A) and DRP-1 transfected (Fig. 4B) COS-7 cells were then fixed and reacted both with Oligreen for nuclear staining and anti-FLAG antibodies for DRP-1 staining. Specific DRP-1 staining was detected in the cytoplasm of these cells, as shown in Figure 4B.

30 A gentle cell extraction was performed with nonionic detergent, 0.5% TRITON X-100, that removes lipids and soluble proteins, leaving intact the detergent insoluble matrix composed of the nucleus, the cytoskeleton framework, and cytoskeleton-

associated proteins. In contrast to DAP-kinase, which is exclusively localized to the cytoskeleton, and hence found only in detergent insoluble fractions (Cohen et al., 1997, Fig. 4C), DRP-1 was preferentially eluted from the detergent soluble fraction, while a small amount was eluted from the insoluble fraction, as shown in Figure 4C. Thus, it was concluded
5 that DRP-1 is a cytoplasmic protein with minor association with insoluble matrix components.

Intrinsic Kinase Activity of DRP-1

To test whether DRP-1 functions as a kinase as predicted from the amino acid sequence, an *in vitro* kinase assay was performed using myosin light chain (MLC) as an
10 exogenous substrate. This substrate was chosen because it is phosphorylated by DAP-kinase (Cohen et al., 1997). DRP-1 was transfected into human kidney 293 cells, immunoprecipitated, and incubated with MLC in the presence and absence of Ca²⁺ and calmodulin. Both MLC phosphorylation and DRP-1 autophosphorylation were evident, as can be seen from Figure 5A.

15 In assaying the *in vitro* kinase activity of DRP-1, the proteins were assayed in the presence or absence of CA2+/CaM and MLC. The proteins were run on 11% SDS-PAGE and blotted to nitrocellulose membrane. Figure 5A shows the autophosphorylation of DRP-1 and MLC phosphorylation, respectively, as seen after exposure of X-ray film. Figure 5B shows the DRP-1 proteins by incubation of the same blot with anti-FLAG antibodies and ECL detection.
20

The addition of Ca2+/calmodulin to the reaction mixture increased the amount of phosphorylated MLC, in accordance with the assumption that, like DAP-kinase, DRP-1 is negatively regulated by the autoinhibitory calmodulin binding domain, and that this inhibition is removed by the binding of Ca2+/calmodulin. A catalytically inactive mutant of DRP-1,
25 DRP-1 K42A, did not phosphorylate MLC and failed to undergo autophosphorylation even though higher amounts of DRP-1 protein were present, as can be seen from Figure 5A. Thus, DRP-1 was found to function *in vitro* as a kinase that is capable of phosphorylating itself and an external substrate. This latter property is stimulated by the addition of Ca2+ and calmodulin.
30

DRP-1 Induces Apoptosis in a Variety of Cell Lines

The high homology to DAP-kinase in the kinase and calmodulin-binding regions suggested the value of checking whether DRP-1 is involved in apoptosis. The wild type DRP-1 and the catalytically inactive mutant of DRP-1, DRP-1 K42A, which are cloned in pCDNA3 vector, were transfected into 293 cells. To quantitate the number of apoptotic cells, 5 these constructs were transfected with a vector expressing the GFP protein. The GFP protein was used as a marker to visualize the transfected cells and to assess the apoptotic frequency among the transfectants according to morphological alterations. Apoptotic cells were scored after 24 hours. Overexpression of the DRP-1 resulted in massive apoptotic cell death (50-60%), as compared to the basal level of apoptotic cells caused by transfection of the 10 non-relevant gene luciferase, shown in Figures 6A-6B and 7.

TOKYO 2004 SEP 6 PCT/US99/13411

Most of the GFP positive green cells rounded up and shrank; some of them showed cytoplasmic blebs, and some were further fragmented into "apoptotic bodies." In addition, some of the transfected cells detached from the plate. This apoptotic cell death was only slightly lower than that of an activated DAP- kinase mutant lacking the autoinhibitory 15 calmodulin regulatory region (ΔCaM; apoptotic values of 70-80%). In contrast, when the cells were transfected with the kinase inactive mutant of DRP-1, DRP-1 K42A, as shown in Figures 6A-6D and 7, no apoptosis was observed. This experiment was repeated six times with reproducible results.

Western blot analysis of transfected cells, using anti- FLAG antibodies, 20 confirmed the expression of both the exogenous wild type and K42A mutant of DRP-1 (Figures 8A and 8B). Similar results were also observed in human SV-80 fibroblasts. In another type of assay, the effect of ectopically expressed DRP-1 on the DNA content of rat embryo primary fibroblasts (REF cells) was assessed, as previously described (Kissil et al., 1999). The REFS were co-transfected with DRP-1 and a membrane-bound form of GFP and 25 then after 48 hours subjected to FACS analysis of their DNA content. A fraction of cells displaying a sub-G1 population, indicative of cells containing fragmented DNA, appeared exclusively in the DRP-1 transfected cells but not in cells transfected with a control vector or with the DRP-1 K42A mutant form. No effect was found on cell cycle distribution of the viable cells.

30 To obtain the results shown in Figures 6A-6D, 1×10^5 293 cells/well were co-transfected with FLAG-tagged wild type DRP-1 or K42A mutant of DRP-1, 1.5 microgram/well and GFP, 0.5 microgram/well. GFP positive cells were visualized under

fluorescent microscope and scored for the appearance of apoptotic morphology 24 hours after transfection. Apoptotic cells are indicated by arrows. The fluorescent microscopic images correspond to 293 cells transfected by pCDNA3-luciferase as negative control (Fig. 6A), pCDNA#-deltaCaM DAP-kinase as positive control (Fig. 6B), pCDNA3-DRP-1 (Fig. 6C), 5 pCDNA3-K42A DRP-1 (Fig. 6D).

In Figure 7, graphs show the percentage of apoptotic cells resulting from the above-mentioned transfections (average \pm S.D. calculated from triplicates of 100 cells each). The scores were taken from the same experiment shown in Figures 6A-6D.

10 In Figures 8A and 8B, proteins extracted from the transfected cells were separated on 10% SDS-PAGE and blotted to nitrocellulose membrane. The blot was hybridized with anti-FLAG antibodies for DRP-1 detection and anti-vinculin antibodies to quantitate the loaded protein amounts. The proteins were prepared from the same experiments shown in Figures 6A-6D.

15 **DAP Kinase Death Domain Protects From DRP-1 Induced Apoptosis**

The structural homology of DRP-1 to DAP-kinase, the common regulation by Ca²⁺/calmodulin, and the finding that both proteins caused apoptosis upon overexpression, suggested that they function along a common apoptotic pathway. In order to test this possibility, the effect of the dominant-negative DAP- kinase death domain (DAPk DD) on 20 DRP-1-induced cell death was analyzed. The laboratory of the present inventor showed recently that overexpression of the fragment encompassing the death domain of DAP-kinase acts as a specific dominant-negative mutant, negating the effects of the full length protein (Datta et al., 1997). As a consequence, it protected cells from TNF-alpha, Fas and FADD/MORT1-induced cell death (Datta et al., 1997).

25 It has now been discovered that DAPk DD protected cell death induced by DRP-1 in 293 cells. As shown in Figure 9A, the apoptotic ratio dropped from 64.3% to 24.7%. A control transfection including DRP-1 and a non-relevant luciferase DNA excluded the possibility that this blockage was simply due to larger amount of DNA used in the transfection. Moreover, the effect of DAPk DD was specific, since the death domain of 30 FADD failed to manifest a similar effect. (Figure 9A). Western blot analysis of transfected cells using anti-FLAG antibodies confirmed the expression of the exogenous DRP-1 in all transfections, as shown in Figure 9B. This experiment was repeated three times with

reproducible results. The ability of the death domain of DAP- kinase to block death induced by DRP-1 implies that DAP-kinase and DRP-1 function along a common pathway.

To obtain the results shown in Figure 9A, 1×10^3 cells/well of 293 cells were co-transfected with 1.2 microgram/well of FLAG-tagged wildtype DRP-1 and 0.5

5 microgram/well of GFP. The scores are the percentage of apoptotic cells given as average \pm S.D. and calculated from triplicates of 100 cells each.

To demonstrate the DRP-1 protein expression in 293 transfected cells shown in Figure 9B, proteins extracted from the transfected cells were separated on 10% SDS-PAGE and blotted to nitrocellulose membrane. The blot was hybridized with anti-FLAG antibodies for DRP-1 detection and anti-vinculin antibodies to quantitate the loaded protein amounts. The proteins were prepared from the same experiment shown in Figure 9A.

Deletion of the C-terminal tail of DRP-1 abolishes its apoptotic activity, while further truncation of the CaM-regulatory region strongly enhances the apoptotic effect

In order to further understand the mode of DRP-1 action in apoptosis, constructs containing C-terminal truncations of DRP-1 tagged by HA were constructed (Fig. 10A). DRP-1 $\Delta 40$ lacks the most C-terminal part of DRP-1 which displays no homology to any known protein. DRP-1 $\Delta 73$ lacks, in addition to that, the CaM-regulatory region of DRP-1, and DRP-1 $\Delta 85$ contains only the catalytic domain. The wild type DRP-1 and the various truncation mutants of DRP-1 were transfected into 293 cells. Induction of apoptotic cell death was assayed as mentioned above in DRP-1 induced apoptosis. Overexpression of the wild type DRP-1 resulted in apoptosis (25%) while the DRP-1 $\Delta 40$ had no effect in these assays. On the other hand, further truncations of the CaM-regulatory region, yielded mutants ($\Delta 73$, $\Delta 85$) which acted as "super-killers" (~90% apoptosis) (Figs. 11A and 11B). This experiment was repeated three times with reproducible results. Western blot analysis of transfected cells, using anti-HA antibodies confirmed the expression of all DRP-1 forms (Fig. 10B). Thus, the dependence of the apoptotic effect of DRP-1 on its kinase activity was confirmed again, since removal of the CaM-regulatory region which acts as an autoinhibitory domain generates a constitutively active kinase. In addition, the existence of a positive module in the C-terminal region of DRP-1, which is necessary for its pro-apoptotic effect, provided that the CaM-regulatory effect is still present, is shown.

The C-terminal part of DRP-1 functions as a homo-dimerization domain

Western analysis performed on proteins extracted from 293 cells transfected by FLAG-tagged DRP-1 revealed an additional band (not shown). This observation led the present inventor to test whether DRP-1 can undergo homo-dimerization. To this aim, two constructs expressing DRP-1 fused to either FLAG or HA tags were co-transfected into 293 cells and classical pull-down experiments with each one of the two epitopes were performed. FLAG-tagged DRP-1 could be shown to bind specifically to HA-tagged DRP-1 in both IP directions (Fig. 12A, see lane 3 in both IP Panels). No binding of DRP-1-HA to FLAG beads or to the irrelevant cytoplasmic protein RFX-ΔSmaI could be observed (Fig. 12A, see IP anti-FLAG panel, lanes 2 or 1+2, respectively). Also non-specific binding of DRP-1-FLAG to HA bead or to RFX-ΔSmaI protein could not be detected (Fig. 12A, see IP anti-HA panel, lanes 1 or 1+2, respectively). Western analyses confirmed the expression of all proteins in these cell extracts (Fig. 12A, see Western panels).

The observation that a C-terminal truncation of 40 amino acids in DRP-1 abolished its apoptotic effect upon ectopic expression in 293 cells, prompted the present inventor to test whether this domain may be involved in the homo-dimerization of DRP-1. DRP-1-FLAG was co-expressed in conjunction with the various deletion mutants of DRP-1 tagged by HA. A strong binding of DRP-1-FLAG to the wild type DRP-1-HA was detected, whereas the binding to DRP-1 Δ40 was mostly abolished (Fig. 12B, upper IP panel, compare lane 1 to 2-4). Western analysis confirmed the expression of wild type DRP-1-HA and all other DRP-1-HA deletion mutants in these transfections (Fig. 12B, see Western panel).

Lower IP panel confirmed the expression of wild type DRP-1-FLAG in all these transfections. Thus, the present inventor concluded that a region spanning the C-terminal 40 amino acids of DRP-1 is responsible for its homo-dimerization. This homo-dimerization is probably required for the apoptotic effect of DRP-1, since DRP-1-Δ40 has lost the ability to induce apoptosis in 293 cells (Figs. 11A and 11B).

Having now fully described this invention, it will be appreciated by those skilled in the art that the same can be performed within a wide range of equivalent parameters, concentrations, and conditions without departing from the spirit and scope of the invention and without undue experimentation.

While this invention has been described in connection with specific embodiments thereof, it will be understood that it is capable of further modifications. This

application is intended to cover any variations, uses or adaptations of the inventions following, in general, the principles of the invention and including such departures from the present disclosure as come within know or customary practice within the art to which the invention pertains and as may be applied to the essential features hereinbefore set forth as follows in the

5 scope of the appended claims.

All references cited herein, including journal articles or abstracts, published or unpublished U.S. or foreign patent application, issued U.S. or foreign patents, or any other references, are entirely incorporated by reference herein, including all data, tables, figures, and text presented in the cited references. Additionally, the entire contents of the references cited

10 within the references cited herein are also entirely incorporated by reference.

Reference to known method steps, conventional method steps, known methods or conventional methods is not in any way an admission that any aspect, description or embodiment of the present invention is disclosed, taught or suggested in the relevant art.

The foregoing description of the specific embodiments will so fully reveal the general nature of the invention that others can, by applying knowledge within the skill of the art (including the contents of the references cited herein), readily modify and/or adapt for various applications such specific embodiments, without undue experimentation, without departing from the general concept of the present invention. Therefore, such adaptations and modifications are intended to be within the meaning and range of equivalents of the disclosed embodiments, based on the teaching and guidance presented herein. It is to be understood that the phraseology or terminology herein is for the purpose of description and not of limitation, such that the terminology or phraseology of the present specification is to be interpreted by the skilled artisan in light of the teachings and guidance presented herein, in combination with the knowledge of one of ordinary skill in the art.

25

10/220-94/67/60
45

REFERENCES

Anderson, P., "Kinase cascades regulating entry into apoptosis," Microbiol. Mo. Biol. Rev., **61**, 33-46, 1997.

Ausubel et al., Current Protocols in Molecular Biology, Green Publications and Wiley
5 Interscience, New York, 1987-1999.

Basu, S., and Kolesnick, R., "Stress signals for apoptosis: ceramide and c-Jun kinase," Oncogene, **17**, 3277-3285, 1998.

Bokoch, G.M., "Caspase-mediated activation of PAK2 during apoptosis: proteolytic kinase activation as a general mechanism of apoptotic signal transduction?", Cell death diff., **5**, 637-645, 1998.

Cardone, M.H., Salveson, G.S., Widmann, C., Johnson, G. And Frisch, S.M., "The regulation of anoikis: MEKK-1 activation requires cleavage by caspases.", Cell, **90**, 315-323, 1997.

Cardone, M.H., Roy, N., Stennicke, H.R., Salvesen, G.S., Franke, T.F., Stanbridge, E., Frisch, S., and Reed, J.S., "Regulation of cell death protease caspase-9 by phosphorylation.", Science, **282**, 318-321, 1998.

Cohen, O., Feinstein, E., and Kimchi, A., "DAP-kinase is a Ca²⁺/calmodulin-dependent, cytoskeletal-associated protein kinase, with cell death-inducing functions that depend on its catalytic activity.", EMBO J., **16**, 998-1008, 1997.

20 Cohen, O., Inbal, B., Kissil, J.L., Feinstein, E., Spivak, T., and Kimchi, A., "DAP-kinase participates in TNF- α and Fas-induced apoptosis and its function requires the death domain.", J. Cell. Biol., in press, 1999.

Datta, S.R., Dudek, H., Tao, X., Masters, S., Fu, H., Gotoh, Y., and Greenberg, M.E., "Akt phosphorylation of BAD couples survival signals to the cell-intrinsic death machinery.", Cell, **91**, 231-241, 1997.

25 Deiss, L.P., Feinstein, E., Berissi, H., Cohen, O., and Kimchi, A., "Identification of a novel serine/threonine kinase and a novel 15-kD protein as potential mediators of the gamma interferon-induced cell death.", Genes Dev., **9**, 15-30, 1995.

Deiss, L.P. and Kimchi, A., "A genetic tool used to identify thioredoxin as a mediator of a growth inhibitory signal.", Science, **252**, 117-120, 1991.

del Peso, L., Gonzalez-Garcia, M., Page, C., Herrera, R., and Nunez, G., "Interleukin-3-induced phosphorylation of BAD through the protein kinase Akt.", *Science*, **282**, 318-321, 1997.

Eshhar, Z. et al., *Br. J. Cancer Suppl.*, **10**, 27-9, 1990.

5 Feinstein, E., Kimchi, A., Wallach, D., Boldin, M., and Varfolomeev, E., "The death domain: a module shared by proteins with diverse cellular functions.", *Trends Biochem. Sci.*, **20**, 342-344, 1995.

Green, D., and Kroemer, G., "The central executioners of apoptosis: caspases or mitochondria?" *Trends Cell Biol.*, **8**, 267-271, 1998.

10 Gross, G. et al., *Proc. Natl. Acad. Sci. USA*, **86**, 10024-8, 1989.

Hanks, S.K., and Quinn, A.M., "Protein kinase catalytic domain sequence database: identification of conserved features of primary structure and classification of family members.", *Methods Enzymol.*, **200**, 38-62, 1991.

Inbal, B., Cohen, O., Polak-Charcon, S., Kopolovic, J., Vadai, E., Eisenbach, L., and Kimchi, 15 A., "DAP kinase links the control of apoptosis to metastasis.", *Nature*, **390**, 180-184, 1997.

Inbal, B., Kissil, J.K., Cohen, O., Spivak-Kroizman, T., and Kimchi, A., "The DAP-related protein kinases-a novel subfamily of serine/threonine kinases with a possible link to apoptosis.", submitted, 1999.

20 Jacobson, M.D., Weil, M., and Raff, M.C., "Programmed cell death in a animal development." *Cell*, **88**, 347-354, 1997.

Kawai, T., Matsumoto, M., Takeda, K., Sanjo, H., and Akira, S., "ZIP kinase, a novel serine/threonine kinase which mediates apoptosis.", *Mol. Cell Biol.*, **18**, 1642-1651, 1998.

Kelliher, M.A., Grimm, S., Ishida, Y., Kuo, F., Stanger, B.Z., and Leder, P., "The death 25 domain kinase RIP mediates the TNF-induced NF- κ B signal.", *Immunity*, **8**, 297-303, 1998.

Kimchi, A., *J. Cell. Biochem.*, **50**, 1-9, 1992.

Kimchi, A., "DAP genes: novel apoptotic genes isolated by a functional approach to gene cloning.", *Biochim. Biophys. Acta*, **1377**, F13-33, 1998.

30 Kissil, J.L., and Kimchi, A., "Death-associated proteins: from gene identification to a the analysis of their apoptotic and tumor suppressive functions.", *Mol. Med. Today*, **4**, 268-74, 1998.

Kissil, J.L., Cohen, O., Raveh, T., and Kimchi, A., "DAP-kinase loss of expression in various carcinoma and B-cell lymphoma cell lines: possible implications for role as tumor suppressor gene.", *EMBO J.*, **18**, 353-362, 1999.

Kogel, D., Plottner, O., Landsberg, G., Christian, S., and Scheidtmann, K.H., "Cloning and characterization of Dlk, a novel serine/threonine kinase that is tightly associated with chromatin and phosphorylates core histones.", *Oncogene*, **17**, 2645-2654, 1998.

Levy et al., *Mol. Cell. Biol.*, **13**, 7942-7952, 1993.

Levy-Strumpf, N., and Kimchi, A., "Death associated proteins (DAPs): from gene identification to the analysis of their apoptotic and tumor suppressive functions." *Oncogene*, **17**, 3331-3340, 1998.

Maundrell, K., Antonsson, B., Magnenat, E., Camps, M., Muda, M., Chabert, C., Gillieron, C., Boschert, U., Vial-Knecht, E., Martinou, J.C., and Artkininstall, S., "Bcl-2 undergoes phosphorylation by c-Jun N-terminal kinase/stress-activated protein kinases in the presence of the constitutively active GTP-binding protein Rac1.", *J. Biol. Chem.*, **272**, 25238-25342, 1997.

McCarthy, J.V., Ni, J., and Dixit, V.M., "RIP2 is a novel NF-kappaB-activating and cell death-inducing kinase.", *J. Biol. Chem.*, **273**, 16968-75, 1998.

Meinkoth et al., *Anal. Biochem.*, **138**, 267-284, 1984.

Park, J., Kim, I., Oh, Y.J., Lee, K., Han, P.L., and Choi, E.J., "Activation of c-Jun N-terminal kinase antagonizes an anti-apoptotic action of Bcl-2.", *J. Biol. Chem.*, **272**, 16725-16728, 1997.

Peitenpol et al, *Cell*, **61**, 777-785, 1990.

Sambrooke et al., *Molecular Cloning: A Laboratory Manual*, 2nd Ed., Cold Spring Harbor Press, Cold Spring Harbor, NY, 1989.

Sanjo, H., Kawai, T., and Akira, S., "DRAKS, novel serine/threonine kinases related to death-associated protein kinase that trigger apoptosis.", *J. Biol. Chem.*, **273**, 29066-29071, 1998.

Stanger, B.Z., Leder, P., Lee, T.H., Kim, E., and Seed, B., "RIP: a novel protein containing a death domain that interacts with Fas/APO-1 (CD95) in yeast and causes cell death.", *Cell*, **81**, 513-523, 1995.

Sun, X., Lee, J., Navas, T., Baldwin, D.T., Stewart, T.A., and Dixit, V.M., "RIP3, a Novel Apoptosis-inducing Kinase.", *J. Biol. Chem.*, **274**, 16871-16875, 1999.

Thompson, J.D., Higgins, D.G., Gibson, T. J., "CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice.", Nucleic Acids Res., **22**, 4673-4680, 1994.

5 Verheij, M., Ruiter, G.A., Zerp, S.F., Van Blitterswijk, W.J., Fuks, Z. Haimovitz-Friedman, A., and Bartelink, H., "The role of the stress-activated protein kinase (SAPK/JNK) signaling pathway in radiation-induced apoptosis.", Radiother Oncol., **47**, 225-232, 1998.

Wahl et al., J Nucl. Med., **24**, 316-325, 1983.

10 White, E., "Life, death and the pursuit of apoptosis.", Genes Dev., **10**, 1-15, 1996.

Yang, X., Khosravi-Far, R., Chang, H.Y., and Baltimore, D., Cell, **89**, 1067-1076, 1997.

Yu, P.W., Huang, B.C., Shen, M., Quast, J., Chan, E., Xu, X., Nolan, G.P., Payan, D.G. and Luo, Y., "Identification of RIP3, a RIP-like kinase that activates apoptosis and Nfkappa B.", Curr. Biol., **9**, 539-42, 1999.

WHAT IS CLAIMED IS:

1. An isolated polypeptide, which is a calmodulin-dependent serine/threonine kinase, or a fragment thereof, selected from the group consisting of:

(A) a polypeptide which is capable of inducing cell death (apoptosis) and comprises

5 the amino acid sequence of SEQ ID NO:2;

(B) a polypeptide which has a property being capable of inducing cell death and has at least 85% sequence identity to the amino acid sequence of SEQ ID NO:2;

(C) a fragment of a polypeptide of SEQ ID NO:2 which is capable of inducing cell death;

10 (D) a fragment which is capable of inducing cell death and has at least 85% sequence identity to fragment (C);

(E) a fragment of a polypeptide of SEQ ID NO:2 which lacks the property of being capable of inducing cell death and which inhibits the ability of polypeptide (A) or (B) to induce cell death; and

(F) a fragment which lacks the property of being capable of inducing cell death and which inhibits the ability of polypeptide (A) or (B) to induce cell death, said fragment having at least 85% sequence identity to fragment (E).

2. An isolated DNA molecule comprising a nucleotide sequence encoding the polypeptide or fragment thereof according to claim 1.

20 3. The isolated DNA molecule according to claim 1, wherein said nucleotide sequence encodes the amino acid sequence of SEQ ID NO:2.

4. The isolated DNA molecule according to claim 3, wherein said nucleotide sequence corresponds to nucleotides 62 to 1141 of SEQ ID NO:1.

00/220-34/67/60
15
20

5. The isolated DNA molecule according to claim 3, which consists of the nucleotide sequence corresponding to nucleotides 62 to 1141 of SEQ ID NO:1.

6. An isolated DNA molecule which hybridizes to the DNA molecule of claim 5 under moderately stringent conditions and encodes a calmodulin-dependent serine/threonine kinase having the property of being capable of inducing cell death.

7. An isolated DNA molecule which hybridizes to the DNA molecule of claim 5 under highly stringent conditions and encodes a calmodulin-dependent serine/threonine kinase having the property of being capable of inducing cell death.

8. A polypeptide consisting of an amino acid sequence selected from the group consisting of amino acid residues 13 to 275 of SEQ ID NO:2 and an amino acid sequence having at least 85% sequence identity to residues 13 to 275 of SEQ ID NO:2.

9. An isolated DNA molecule comprising a nucleotide sequence encoding the polypeptide of claim 8.

10. The isolated DNA molecule according to claim 9, wherein said nucleotide sequence encodes the amino acid sequence corresponding to residues 13 to 275 of SEQ ID NO:2.

11. The isolated DNA molecule according to claim 10, wherein said nucleotide sequence is selected from the group consisting of nucleotides 98 to 886 of SEQ ID NO:1 and a nucleotide sequence which hybridizes to nucleotides 98 to 886 of SEQ ID NO:1 under moderately stringent conditions.

12. The isolated DNA molecule according to claim 11, wherein said nucleotide sequence hybridizes to nucleotides 98 to 886 of SEQ ID NO:1 under highly stringent conditions.

20 25 30 35 40 45 50

13. A polypeptide consisting of an amino acid sequence selected from the group consisting of amino acid residues 321 to 360 of SEQ ID NO:2 and an amino acid sequence having at least 85% sequence identity to residues 321 to 360 of SEQ ID NO:2.

14. An isolated DNA molecule comprising a nucleotide sequence encoding the 5 polypeptide of claim 13.

15. The isolated DNA molecule according to claim 14, wherein said nucleotide sequence encodes the amino acid sequence corresponding to residues 321 to 360 of SEQ ID NO:2.

16. The isolated DNA molecule according to claim 15, wherein said nucleotide 10 sequence is selected from the group consisting of nucleotides 1022 to 1141 of SEQ ID NO:1 and a nucleotide sequence which hybridizes to nucleotides 1022 to 1141 of SEQ ID NO:1 under moderately stringent conditions.

17. The isolated DNA molecule according to claim 16, wherein said nucleotide sequence hybridizes to nucleotides 1022 to 1141 of SEQ ID NO:1 under highly stringent conditions.

18. A vector comprising the isolated DNA molecule according to any of claims 2-7, 9-12 and 14-17.

19. A host cell transformed with the isolated DNA molecule according to any of claims 2-7, 9-12, and 14-17.

20. A composition comprising a polypeptide according to any one of claims 1, 8 and 13, and a pharmaceutically acceptable excipient, carrier, diluent or auxiliary agent.

21. A molecule containing an antigen binding portion of an antibody which specifically recognizes the polypeptide according to any one of claims 1, 8 and 13 with the proviso that said antibody does not cross-react with DAP kinase or ZIP kinase.

25 22. The antibody according to claim 21, which is a monoclonal antibody.

23. A single stranded RNA molecule complementary to at least a portion of the isolated messenger RNA molecule which is the transcription product of the DNA sequence encoding a polypeptide of SEQ ID NO:2, wherein said complementary single stranded RNA molecule is capable of hybridizing to said isolated messenger RNA to prevent its translation 30 into said polypeptide of SEQ ID NO:2.

24. A method of neutralizing a messenger RNA molecule, which is the transcription product of the DNA sequence encoding a polypeptide of SEQ ID NO:2, comprising the step

1022-084/67/60
20

of contacting the single stranded RNA molecule of claim 23 with the messenger RNA to neutralize the messenger RNA by hybridizing thereto and preventing its translation into the polypeptide of SEQ ID NO:2.

25. A method for screening individuals for a predisposition to cancer comprising the

5 steps of:

(a) obtaining a sample of either genomic DNA from cells of the individual or cDNA produced from mRNA of said cells; and

(b) determining if there is a mutation in the nucleotide sequence of the DRP-1 gene.

26. The method according to claim 25 wherein a mutation in the nucleotide sequence 10 of DRP-1 is determined by a process comprising the steps of:

(a) adding one or more nucleic acid probes to the sample of genomic DNA or cDNA, wherein each probe comprises a portion of the nucleotide sequence of DRP-1;

(b) providing conditions for hybridization between the nucleic acid probe or probes and the DNA of said samples; and

(c) determining on the basis of the hybridization whether there is a match between the sequence of the nucleic acid probe or probes and a sequence in the DNA of said sample, or whether there is a mismatch, a deletion or a mutation in the genomic DNA or cDNA and a predisposition to cancer of the tested individual.

15
16
17
18
19
20

FIG. 1

GACCGCGGCAGCTCAGCCTCCGCCGATTGTATGTTCCAGGCCTCAATGAGGAGTCCAAA
 M E P F K Q Q K V E D F Y D I G E E L G 60
 C~~ATG~~ GAGCCATTCAAGCAGCAGAAGGTGGAGGACTTTATGACATCGAGAGGAGCTGGG 20
 S G Q F A I V K K C R E K S T G L E Y A 40
 GAGTGGCCAGTTGCCATCGTAAGAAGTGCAGGGAGAAGAGCAGGGCTTGAGTATGC 120
 A K F I K K R Q S R A S R R G V S R E E 180
 AGCCAAGTTCATCAAGAAGCAGCAGAGCGGGCGAGGCCGGCGCGGTGTGAGCCGGAGGA 60
 I E R E V S I L R Q V L H H N V I T L H 240
 GATCGAGCGGGAGGTGAGCATCTCGGGCAGGTGACCACAAATGTCATCAGCTGCA 80
 D V Y E N R T D V V L I L E L V S G G E 300
 CGACGCTCATGAGAACCGCACCGACGTGGTGCTCATCCTTGAGCTAGTGTCTGGAGGAGA 100
 L L F D F L A Q K E S L S S E E E A T S F I 360
 GCTCTCGATTTCTGGCCCAGAAGGAGTCACTGAGTGGAGGAGGAGGCCACAGCTTCAT 120
 K Q I L D G V N Y L H T K K I A H F D L 420
 TAAGCAGATCTGGATGGGTGAACCTACACAAAGAAAATTGCTCACTTGATCT 140
 K P E N I M L L D K N I P I P H I K L I 480
 CAAGCAGAAACATTATGTTGTTAGACAAGAATATTCCCATTCACACATCAAGCTGAT 160
 D F G L A H E I E D G V E F K N I F G T 540
 TGACTTTGGTCTGGCTCACGAAATAGAAGATGAGGTGAATTAAAGAATTTTGGGAG 180
 P E F V A P E I V N Y E P L G L E A D M 600
 CGCCGAATTGTTGTCAGAAATTGTAAGACTACAGAGCCCTGGGTCTGGAGGCTGACAT 200
 W S I G V I T Y I L L S G A S P F L G D 660
 GTGGAGCATAGGCGTCATCACCACATCCTTAAGTGGAGCATCCCTTCTGGAGA 220
 T K Q E T L A N I T S V S Y D F D E E F 720
 CACGAAGCAGGAAACACTGGCAAATATCACATCAGTGAGTTACGACTTTGATGAGGAATT 240
 F S H T S E L A K D F I R K L L V K E T 780
 CTTCGCCATACGAGCGAGCTGGCAAGGACTTTATCGGAAGGACTCTGGTTAACAGAC 260
 R K R L T I Q E A L R H P W I T P V D N 840
 CCGGAAACGGCTCACAACTCAAGAGGCTCTCAGACACCCCTGGATCACGCCGGTGGACAA 280
 Q Q A M V R R E S V V N L E N F R K Q Y 900
 CCAGCAAGGCTATGGTGGCCAGGGAGTCTGTGGTCAATCTGGAGAACCTCAGGAAGCAGTA 300
 V R R R W K L S F S I V S L C N H L T R 960
 TGTCGGCAGGGCTGGAAAGCTTCTTCAGCTCGTGTCCCTGTGCAACCCACCTCACCCG 320
 S L M K V H L R P D E D L R N C E S D 1020
 CTCGCTGATGAAGAAGGTGCACCTGAGGCCGGATGAGGACCTGAGGAAGTGTGAGAGTGA 340
 T E E D I A A R R K A L H P R R S S T S 1080
 CACTGAGGAGGACATGCCAGGAGGAAGGCCCTCCACCCACGGAGGAGGAGCAGCACCTC 360
 C~~TAAC~~ TGCCCTGACCTCGAGTGGCCGCAGGGAGGTTGGGCCAGGGGGCTCCCTCT 1140
 GTGCAGACTTTGGACCCAGCTCAGCACCCAGCACCCGGCGTCTGAGCACTTGCAAGA 1200
 GAGATGGGGCCAAAGGAATTCAGAGAGACTTGCGAGGACAGGAGACCTTGGAGCTGT 1260
 GGCCTGCTTCCTGGAGGAGGCTCCAGCATTCCCAAAGCTCTTAAATTCTCATAAAATGG 1320
 GCTTCTCTGTCTGCCATCCTCAGACTGGGGTGGAGTGTGGACTTAGGAAAACAT 1380
 ATAAAGGACATCTCATCATCGAGGTTGAAGGTCAGAGTAAAGCAGCCTTCTCAGG 1440
 CTGAGGGGGTTCAGAACCCAGGCCAAATTACACAGAGAGACAGTCCCTCCCA 1500
 TTGGGAACAGGGTGAATTGAGGAAAGTGAACCTTgGGTGTGAGGGACCAATCCTGTGACCT 1560
 CCCAGAACCATGGAAGCAGGACGTCAGGCTGAACACACCTCAGACACCTCTGAAGCAGC 1620
 CCATTGCTGGCCCGCCATGTTGTAATTTCGTCATTTTATTAAActctggttacctg 1680
 aa 1740

T0220-34267460

FIG. 2A

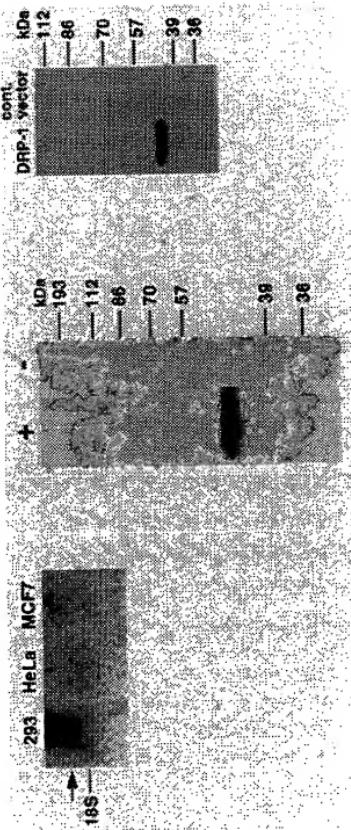
DAP-kinase	13	YDTGEELGSGQFAVVKCREKSTGLOYAAKFIK
ZIP-kinase	13	YEMGEELGSGQFAVVKCQKGTGKEYAAKFIK
DRP-1	13	YDGEELGSGQFAVVKCREKSTGLEYAAKFIK
DRAK1	61	LCPGRELGRGEFAVVRKCIKKDSGKEFAAKFMR
DRAK2	33	ILTSKELGRGEFAVVRQCIISKSTGQYAAKFIK
DAP-kinase	46	KRRTKSSRRGVSRDIEREVSIILKEI-QHENVI
ZIP-kinase	46	KRRLSSSRGVSRREEIEREVNLREI-RHPNII
DRP-1	46	KRDSRASRRGVSRREEIEREVSIILRQV-LHHNVI
DRAK1	94	KRR----KGQDORMEIIHEIAVLELAQDNPWNVI
DRAK2	66	KRR----KGQDORAEILHEIAVLELAKSOPRVI
DAP-kinase	78	TLHEVYENKTDVVLILELVAGGELFD-FLAEC-
ZIP-kinase	78	TLHDIEENKTDVVLILELVSGGELFD-FLAEC-
DRP-1	78	TLHDVYENKTDVVLILELVSGGELFD-FLAEC-
DRAK1	123	NLHEVYETASEMILVLEYAAGGEIFDQCVDRE
DRAK2	95	NLHEVYENTSEIILILEYAAAGGEIFSLCLPELA
DAP-kinase	109	ESLTSEEATEFLKQIILNGVYLYHSLQIAHEDLK
ZIP-kinase	109	ESLTEDEATQFLKQIILDGVHYLESRIAHEDLK
DRP-1	109	ESLSEEATSFQFLKQIILDGVNLYLHTKKIAHEDLK
DRAK1	156	EAFFKEKDQVQLMRQILEGVHFLHTRDVVHEDLK
DRAK2	128	EMVSENDVIRLIKQIILEGVYYLHQNNVHEDLK
DAP-kinase	142	PENIILLLDRNVPKPRIKIDFGLAHKIDFGNEF
ZIP-kinase	142	PENIILLLDKNVPNPRIKIDFGIAHKDNEAGNEF
DRP-1	142	PENIILLLDKNPIPIHKLIDFGGLAHEIDFGVEF
DRAK1	189	PQNIILLTSSEPLGD-IKIVDFGLSRLKNEEL
DRAK2	161	PQNIILLSSIYPLGD-IKIVDFGMSRKQGHACEL
DAP-kinase	175	KNIEGTPEFVAPEIIVNYEPLGLEADMWSIGVIT
ZIP-kinase	175	KNIEGTPEFVAPEIIVNYEPLGLEADMWSIGVIT
DRP-1	175	KNIEGTPEFVAPEIIVNYEPLGLEADMWSIGVIT
DRAK1	221	REIMGTPEYVAPEILSYDPIISMATDMWSIGVLT
DRAK2	193	REIMGTPEYVAPEILNYDPIITTATDMWNLIGHT
DAP-kinase	208	YILLSGASPFLGDTKQETLANISAVNYEFDEEY
ZIP-kinase	208	YILLSGASPFLGETKQETLNISAVNYDFDEEY
DRP-1	208	YILLSGASPFLGDTKQETLANITSVSYDFDEEY
DRAK1	254	YVMLTGTISPFLGNDKQETFLNISOMNLSSYSEE
DRAK2	226	YVMLTHTSPFVGEDNQETYLNISQVNVDYSEET
DAP-kinase	241	FSNTSALAKDFIRRLLVKDPKRRMTIQDSLQHP
ZIP-kinase	241	FSNTSELAKDFIRRLLVKDPKRRMTIAQSLEHS
DRP-1	241	FSHTSELAKDFIRRLLVKETRKRLTIQEALRHP
DRAK1	287	FDVLSESAVDFIRRLLVKDPEDRATAEECLKHP
DRAK2	259	FSSVSQLATDFQSSLVKNPEKRPTAEICLHS
DAP-kinase	274	WI
ZIP-kinase	274	WI
DRP-1	274	WI
DRAK1	320	WL
DRAK2	292	WL

FIG. 2B

DAP-kinase	1	NMEK KK ---F MARKK WQSVRLISLC Q LSR	29
DRP-1	1	NLEN FP ---QY Y RRRW KL SFSIV S LC N HLTR	29
smMLCK	1	SKDRM KK ---Y M ARRK W QK T GHAVRAIC R LSS	29
CaMKIIa	1	TVDC KK ---L N ARRK W Q K GAILTTM L ATRNFS	29
CaMKI	1	VSEQ IKK ---NF A RS K W K QAFNAT- A V V RHMR	28
CaMKIV	1	MDTACK KK Q E FN A RRK L KA V AVV V ASS R LGS	32
ZIP-kinase	1	GEDSG R ---P E RRRL K PPRLKEYTIKSHSS	28

T00220 * 24.297262

FIG. 3A FIG. 3B FIG. 3C



5 / 16

FIG. 4A

MOCK

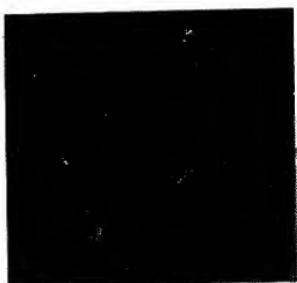
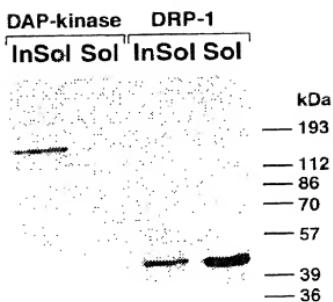


FIG. 4B

DRP-1



FIG. 4C



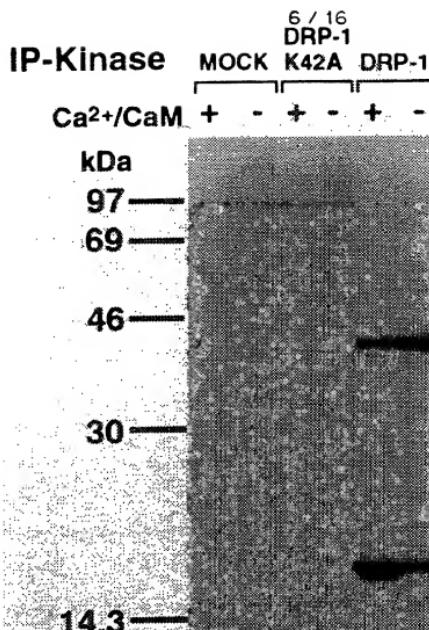
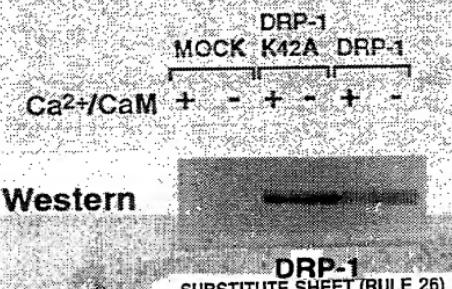


FIG. 5A

FIG. 5B

DRP-1
SUBSTITUTE SHEET (RULE 26)

7 / 16

FIG. 6B

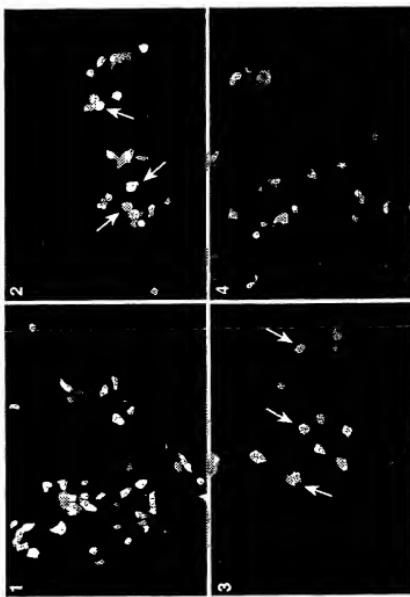
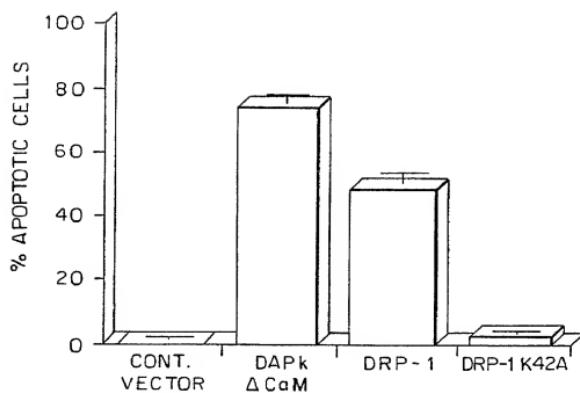


FIG. 6D

FIG. 6A

FIG. 6C

FIG. 7



9 / 16

FIG. 8A

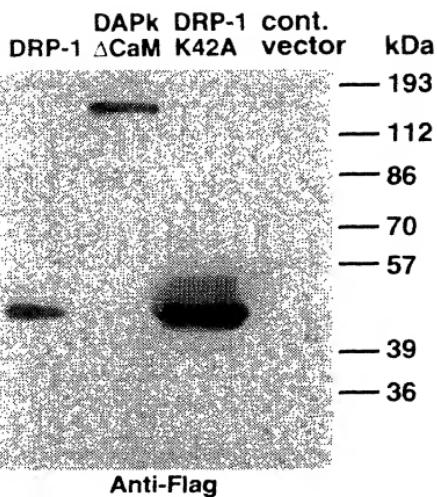


FIG. 8B

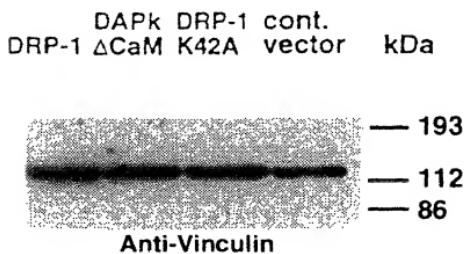


FIG. 9A

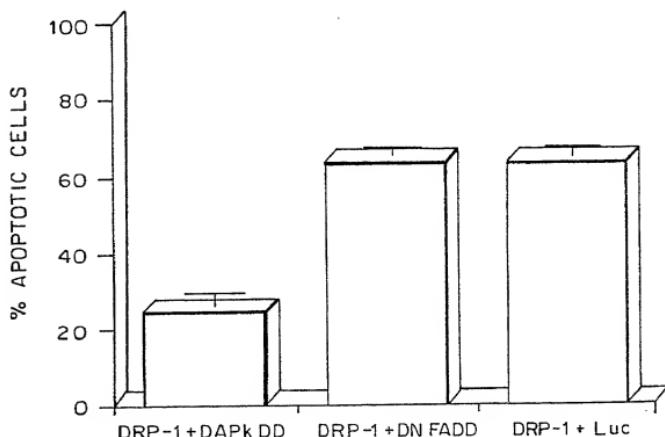


FIG. 9B

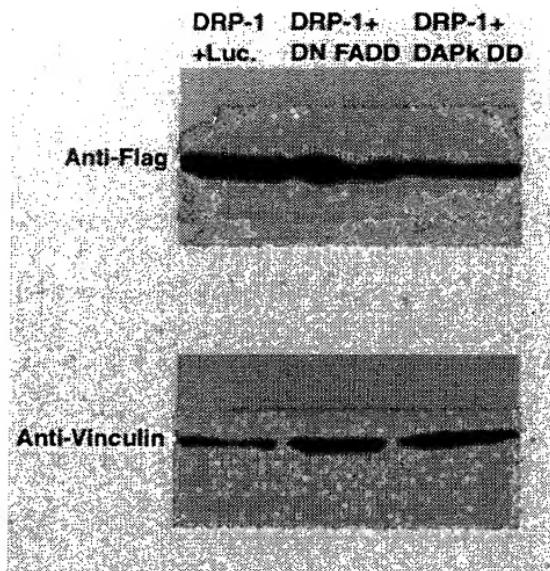


FIG. 10A

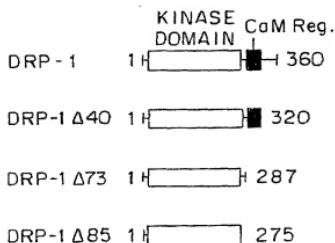
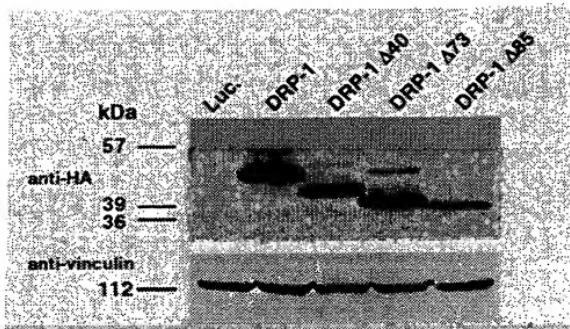


FIG. 10B



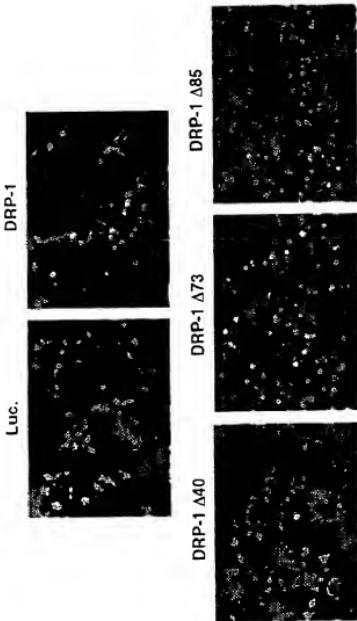
F/G. 11A

FIG. 11B

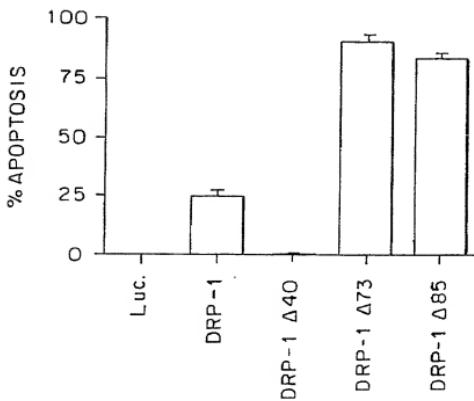
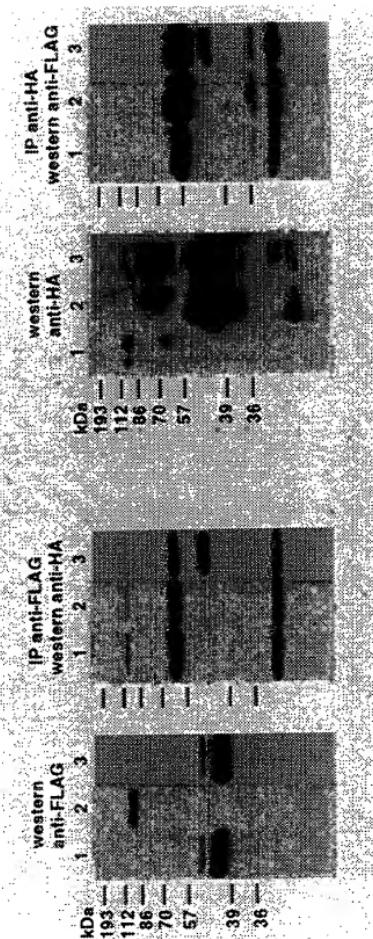
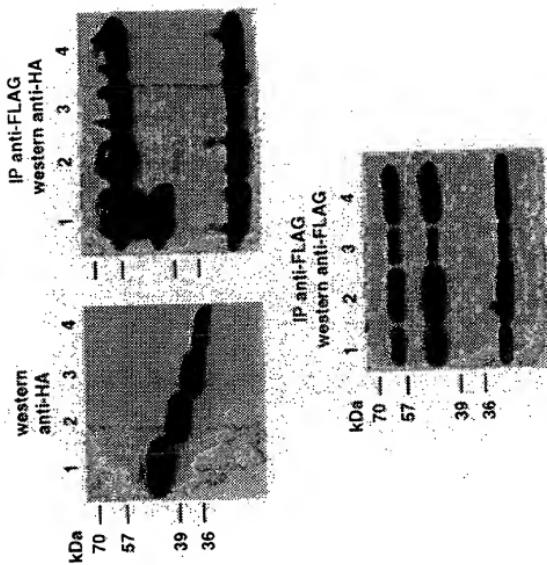


FIG. 12A



16 / 16

FIG. 12B



Page 1 of 2 Pages Original Substitute Supplemental Any Docket: KIMCHI=2A**Combined Declaration for Patent Application and Power of Attorney**

As a below-named inventor, I hereby declare that
 My residence, post office address and citizenship are as stated below next to my name; and that I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled:

DAP KINASE RELATED PROTEIN

the specification of which (check one)

is attached hereto;
 was filed in the United States under 35 U.S.C. §111 on _____, as
 U.S. Appln. No. _____, or
 was filed in the U.S. under 35 U.S.C. §371 by entry into the U.S. national stage of an international (PCT) application, PCT/US99/13411, filed June 15, 1999, entry requested on December 15, 2000*, national stage application received U.S. Appln. No. 09/719,748, §371/§102(e) date: _____ (* if known)

and was amended on _____ (if applicable).

(include date of amendment under PCT Art. 19 and 34 of PCT)

I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above, and I acknowledge the duty to disclose to the Patent and Trademark Office (PTO) all information known by me to be material to patentability as defined in 37 C.F.R. §1.56.

I hereby claim foreign priority benefits under 35 U.S.C. §§ 119 (a)-(d) and 365 (b) of any prior foreign application(s) for patent or inventor's certificate, or §365(a) of any prior PCT application(s) designating a country other than the U.S., listed below with the "Yes" box checked, and have also identified below, by checking the "No" box, any foreign application for patent or inventor's certificate or PCT international application having a filing date before that of the application on which priority is claimed:

(Number)	(Country)	(Day Month Year Filed)	YES	NO
(Number)	(Country)	(Day Month Year Filed)	YES	NO

I hereby claim the benefit under 35 U.S.C. §119(e) of any United States provisional applications listed below:

60/089,294 (Application No.)	15 June 1998 (Day Month Year Filed)
(Application No.)	(Day Month Year Filed)

I hereby claim the benefit under 35 U.S.C. §120 of any prior U.S. non-provisional application(s) or under §365(e) of any prior PCT international application(s) designating the U.S., listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in such U.S. or PCT international application, in the manner provided by the first paragraph of 35 U.S.C. §121, I acknowledge the duty to disclose to the PTO all information which is material to patentability as defined in 37 C.F.R. §1.56 which became available between the filing date of the prior application and the national or PCT international filing date of this application:

(Application No.)	(Day Month Year Filed)	(Status: patented, pending, abandoned)
(Application No.)	(Day Month Year Filed)	(Status: patented, pending, abandoned)

As a named inventor, I hereby appoint the following registered practitioners to prosecute this application and to transact all business in the Patent and Trademark Office connected therewith:

All of the practitioners associated with Customer Number 001444

Direct all correspondence to the address associated with Customer Number 001444, which is presently:

BROWNDY AND NEWMARK, P.L.L.C.
 624 Ninth Street, N.W.
 Washington, D.C. 20001-3303
 (202) 628-5197

The undersigned hereby authorizes the U.S. Attorneys or Agents appointed herein to accept and follow instructions from Yeda Research and Development Co. Ltd. as to any action to be taken in the U.S. Patent and Trademark Office regarding this application without direct communication between the U.S. Attorneys or Agents and the undersigned. In the event of a change of the persons from whom instructions may be taken, the U.S. Attorneys or Agents appointed herein will be so notified by the undersigned.

Page 2 of 2 Pages

Title: DAP-KINASE RELATED PROTEIN

U.S. Application filed

Serial No.

PCT Application filed June 15, 1999Serial No. ECT/US99/13411

Any Docket: KIMCHI=2A

I hereby further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true, and that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under 18 U.S.C. §1001 and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

FULL NAME OF FIRST INVENTOR <u>Adi KIMCHI</u>		INVENTOR'S SIGNATURE <u>Adi Kimchi</u>	DATE <u>8/26/01</u>
RESIDENCE <u>Ramana, ISRAEL</u>	CITIZENSHIP <u>Israel</u>		
POST OFFICE ADDRESS <u>38 Hashalom Street, 43561 Ramana, Israel</u>			
FULL NAME OF SECOND JOINT INVENTOR		INVENTOR'S SIGNATURE	DATE
RESIDENCE	CITIZENSHIP		
POST OFFICE ADDRESS			
FULL NAME OF THIRD JOINT INVENTOR		INVENTOR'S SIGNATURE	DATE
RESIDENCE	CITIZENSHIP		
POST OFFICE ADDRESS			
FULL NAME OF FOURTH JOINT INVENTOR		INVENTOR'S SIGNATURE	DATE
RESIDENCE	CITIZENSHIP		
POST OFFICE ADDRESS			
FULL NAME OF FIFTH JOINT INVENTOR		INVENTOR'S SIGNATURE	DATE
RESIDENCE	CITIZENSHIP		
POST OFFICE ADDRESS			
FULL NAME OF SIXTH JOINT INVENTOR		INVENTOR'S SIGNATURE	DATE
RESIDENCE	CITIZENSHIP		
POST OFFICE ADDRESS			
FULL NAME OF SEVENTH JOINT INVENTOR		INVENTOR'S SIGNATURE	DATE
RESIDENCE	CITIZENSHIP		
POST OFFICE ADDRESS			

ALL INVENTORS MUST REVIEW APPLICATION AND DECLARATION BEFORE SIGNING. ALL ALTERNATIVES MUST BE INITIALED AND SIGNED BY ALL INVENTORS PRIOR TO EXECUTION.
NO ALTERNATIVES CAN BE MADE AFTER THE DECLARATION IS SIGNED. ALL PARTS OF DECLARATION MUST BE SIGNED BY ALL INVENTORS.

Replaced

Rec'd PCT/PTC 20 APR 2001

SEQUENCE LISTING

<110> KIMCHI, Adi

<120> DAP-KINASE RELATED PROTEIN

<130> KIMCHI2A

<140> 09/719,748

<141> 2000-12-15

<150> 60/089,294

<151> 1998-06-15

<160> 14

<170> PatentIn Ver. 2.0

<210> 1

<211> 1742

<212> DNA

<213> Human

<220>

<221> CDS

<222> (62)..(1141)

<400> 1

gaccgcggca gctcaggcctc ccggccgattt tatgttccag gcctcaatga ggagtccaaa 60

c atg gag cca ttc aag gag aag gtg gag gac ttt tat gac atc gga 109
Met Glu Pro Phe Lys Gln Gln Lys Val Glu Asp Phe Tyr Asp Ile Gly
1 5 10 15

gag gag ctg ggg agt ggc cag ttt gcc atc gtg aag aag tgc cgg gag 157
Glu Glu Leu Gly Ser Gly Gln Phe Ala Ile Val Lys Lys Cys Arg Glu
20 25 30

aag agc acg ggg ctt gag tat gca gcc aag ttc atc aag aag cgg cag 205
Lys Ser Thr Gly Leu Glu Tyr Ala Ala Lys Phe Ile Lys Lys Arg Gln
35 40 45

agc cgg gcg agc cgg cgc ggt gtg agc cgg gag gag atc gag cgg gag 253
Ser Arg Ala Ser Arg Arg Gly Val Ser Arg Glu Glu Ile Glu Arg Glu
50 55 60

gtg agc atc ctg cgg cag gtg ctg cac cac aat gtc atc acg ctg cac 301
Val Ser Ile Leu Arg Gln Val Leu His His Asn Val Ile Thr Leu His
65 70 75 80

gac gtc tat gag aac cgc acc gac gtg gtg cac atc ctt gag cta gtg 349
Asp Val Tyr Glu Asn Arg Thr Asp Val Val His Ile Leu Glu Leu Val
85 90 95

tct gga gga gag ctc ttc gat ttc ctg gcc cag aag gag tca ctg agt 397
Ser Gly Gly Glu Leu Phe Asp Phe Leu Ala Gln Lys Glu Ser Leu Ser
100 105 110



1637220 2001-04-20

gag gag gag gcc acc agc ttc att aag cag atc ctg gat ggg gtg aac	445																																																																																																																
Glu Glu Glu Ala Thr Ser Phe Ile Lys Gln Ile Leu Asp Gly Val Asn																																																																																																																	
115	120		125	tac ctt cac aca aag aaa att gct cac ttt gat ctc aag cca gaa aac	493	Tyr Leu His Thr Lys Lys Ile Ala His Phe Asp Leu Lys Pro Glu Asn		130	135		140	att atg ttg tta gac aag aat att ccc att cca cac atc aag ctg att	541	Ile Met Leu Leu Asp Lys Asn Ile Pro His Ile Lys Leu Ile		145	150		155		160	gac ttt ggt ctg gct cac gaa ata gaa gat gga gtt gaa ttt aag aat	589	Asp Phe Gly Leu Ala His Glu Ile Glu Asp Gly Val Glu Phe Lys Asn		165	170		175	att ttt ggg acg ccg gaa ttt gtt gct cca gaa att gtg aac tac gag	637	Ile Phe Gly Thr Pro Glu Phe Val Ala Pro Glu Ile Val Asn Tyr Glu		180	185		190	ccc ctg ggt ctg gag gct gac atg tgg agc ata ggc gtc atc acc tac	685	Pro Leu Gly Leu Glu Ala Asp Met Trp Ser Ile Gly Val Ile Thr Tyr		195	200		205	atc ctc tta agt gga gca tcc cct ttc ctg gga gac acg aag cag gaa	733	Ile Leu Leu Ser Gly Ala Ser Pro Phe Leu Gly Asp Thr Lys Gln Glu		210	215		220	aca ctg gca aat atc aca tca gtg agt tac gac ttt gat gag gaa ttc	781	Thr Leu Ala Asn Ile Thr Ser Val Ser Tyr Asp Phe Asp Glu Glu Phe		225	230		235		240	ttc agc cat acg agc gag ctg gcc aag gac ttt att cgg aag ctt ctg	829	Phe Ser His Thr Ser Glu Leu Ala Lys Asp Phe Ile Arg Lys Leu Leu		245	250		255	gtt aaa gag acc ccg aaa ccg ctc aca atc caa gag gct ctc aga cac	877	Val Lys Glu Thr Arg Lys Arg Leu Thr Ile Gln Glu Ala Leu Arg His		260	265		270	ccc tgg atc acg ccg gtg gac aac cag caa gcc atg gtg cga ccg gag	925	Pro Trp Ile Thr Pro Val Asp Asn Gln Gln Ala Met Val Arg Arg Glu		275	280		285	tct gtg gtc aat ctg gag aac ttc agg aag cag tat gtc cgc agg ccg	973	Ser Val Val Asn Leu Glu Asn Phe Arg Lys Gln Tyr Val Arg Arg Arg		290	295		300	tgg aag ctt tcc ttc agc atc gtg tcc ctg tgc aac cac ctc acc cgc	1021	Trp Lys Leu Ser Phe Ser Ile Val Ser Leu Cys Asn His Leu Thr Arg		305	310		315		320	tcg ctg atg aag aag gtg cac ctg agg ccg gat gag gac ctg agg aac	1069	Ser Leu Met Lys Lys Val His Leu Arg Pro Asp Glu Asp Leu Arg Asn		325	330		335
	125																																																																																																																
tac ctt cac aca aag aaa att gct cac ttt gat ctc aag cca gaa aac	493																																																																																																																
Tyr Leu His Thr Lys Lys Ile Ala His Phe Asp Leu Lys Pro Glu Asn																																																																																																																	
130	135		140	att atg ttg tta gac aag aat att ccc att cca cac atc aag ctg att	541	Ile Met Leu Leu Asp Lys Asn Ile Pro His Ile Lys Leu Ile		145	150		155		160	gac ttt ggt ctg gct cac gaa ata gaa gat gga gtt gaa ttt aag aat	589	Asp Phe Gly Leu Ala His Glu Ile Glu Asp Gly Val Glu Phe Lys Asn		165	170		175	att ttt ggg acg ccg gaa ttt gtt gct cca gaa att gtg aac tac gag	637	Ile Phe Gly Thr Pro Glu Phe Val Ala Pro Glu Ile Val Asn Tyr Glu		180	185		190	ccc ctg ggt ctg gag gct gac atg tgg agc ata ggc gtc atc acc tac	685	Pro Leu Gly Leu Glu Ala Asp Met Trp Ser Ile Gly Val Ile Thr Tyr		195	200		205	atc ctc tta agt gga gca tcc cct ttc ctg gga gac acg aag cag gaa	733	Ile Leu Leu Ser Gly Ala Ser Pro Phe Leu Gly Asp Thr Lys Gln Glu		210	215		220	aca ctg gca aat atc aca tca gtg agt tac gac ttt gat gag gaa ttc	781	Thr Leu Ala Asn Ile Thr Ser Val Ser Tyr Asp Phe Asp Glu Glu Phe		225	230		235		240	ttc agc cat acg agc gag ctg gcc aag gac ttt att cgg aag ctt ctg	829	Phe Ser His Thr Ser Glu Leu Ala Lys Asp Phe Ile Arg Lys Leu Leu		245	250		255	gtt aaa gag acc ccg aaa ccg ctc aca atc caa gag gct ctc aga cac	877	Val Lys Glu Thr Arg Lys Arg Leu Thr Ile Gln Glu Ala Leu Arg His		260	265		270	ccc tgg atc acg ccg gtg gac aac cag caa gcc atg gtg cga ccg gag	925	Pro Trp Ile Thr Pro Val Asp Asn Gln Gln Ala Met Val Arg Arg Glu		275	280		285	tct gtg gtc aat ctg gag aac ttc agg aag cag tat gtc cgc agg ccg	973	Ser Val Val Asn Leu Glu Asn Phe Arg Lys Gln Tyr Val Arg Arg Arg		290	295		300	tgg aag ctt tcc ttc agc atc gtg tcc ctg tgc aac cac ctc acc cgc	1021	Trp Lys Leu Ser Phe Ser Ile Val Ser Leu Cys Asn His Leu Thr Arg		305	310		315		320	tcg ctg atg aag aag gtg cac ctg agg ccg gat gag gac ctg agg aac	1069	Ser Leu Met Lys Lys Val His Leu Arg Pro Asp Glu Asp Leu Arg Asn		325	330		335								
	140																																																																																																																
att atg ttg tta gac aag aat att ccc att cca cac atc aag ctg att	541																																																																																																																
Ile Met Leu Leu Asp Lys Asn Ile Pro His Ile Lys Leu Ile																																																																																																																	
145	150		155		160	gac ttt ggt ctg gct cac gaa ata gaa gat gga gtt gaa ttt aag aat	589	Asp Phe Gly Leu Ala His Glu Ile Glu Asp Gly Val Glu Phe Lys Asn		165	170		175	att ttt ggg acg ccg gaa ttt gtt gct cca gaa att gtg aac tac gag	637	Ile Phe Gly Thr Pro Glu Phe Val Ala Pro Glu Ile Val Asn Tyr Glu		180	185		190	ccc ctg ggt ctg gag gct gac atg tgg agc ata ggc gtc atc acc tac	685	Pro Leu Gly Leu Glu Ala Asp Met Trp Ser Ile Gly Val Ile Thr Tyr		195	200		205	atc ctc tta agt gga gca tcc cct ttc ctg gga gac acg aag cag gaa	733	Ile Leu Leu Ser Gly Ala Ser Pro Phe Leu Gly Asp Thr Lys Gln Glu		210	215		220	aca ctg gca aat atc aca tca gtg agt tac gac ttt gat gag gaa ttc	781	Thr Leu Ala Asn Ile Thr Ser Val Ser Tyr Asp Phe Asp Glu Glu Phe		225	230		235		240	ttc agc cat acg agc gag ctg gcc aag gac ttt att cgg aag ctt ctg	829	Phe Ser His Thr Ser Glu Leu Ala Lys Asp Phe Ile Arg Lys Leu Leu		245	250		255	gtt aaa gag acc ccg aaa ccg ctc aca atc caa gag gct ctc aga cac	877	Val Lys Glu Thr Arg Lys Arg Leu Thr Ile Gln Glu Ala Leu Arg His		260	265		270	ccc tgg atc acg ccg gtg gac aac cag caa gcc atg gtg cga ccg gag	925	Pro Trp Ile Thr Pro Val Asp Asn Gln Gln Ala Met Val Arg Arg Glu		275	280		285	tct gtg gtc aat ctg gag aac ttc agg aag cag tat gtc cgc agg ccg	973	Ser Val Val Asn Leu Glu Asn Phe Arg Lys Gln Tyr Val Arg Arg Arg		290	295		300	tgg aag ctt tcc ttc agc atc gtg tcc ctg tgc aac cac ctc acc cgc	1021	Trp Lys Leu Ser Phe Ser Ile Val Ser Leu Cys Asn His Leu Thr Arg		305	310		315		320	tcg ctg atg aag aag gtg cac ctg agg ccg gat gag gac ctg agg aac	1069	Ser Leu Met Lys Lys Val His Leu Arg Pro Asp Glu Asp Leu Arg Asn		325	330		335																
	155		160	gac ttt ggt ctg gct cac gaa ata gaa gat gga gtt gaa ttt aag aat	589	Asp Phe Gly Leu Ala His Glu Ile Glu Asp Gly Val Glu Phe Lys Asn		165	170		175	att ttt ggg acg ccg gaa ttt gtt gct cca gaa att gtg aac tac gag	637	Ile Phe Gly Thr Pro Glu Phe Val Ala Pro Glu Ile Val Asn Tyr Glu		180	185		190	ccc ctg ggt ctg gag gct gac atg tgg agc ata ggc gtc atc acc tac	685	Pro Leu Gly Leu Glu Ala Asp Met Trp Ser Ile Gly Val Ile Thr Tyr		195	200		205	atc ctc tta agt gga gca tcc cct ttc ctg gga gac acg aag cag gaa	733	Ile Leu Leu Ser Gly Ala Ser Pro Phe Leu Gly Asp Thr Lys Gln Glu		210	215		220	aca ctg gca aat atc aca tca gtg agt tac gac ttt gat gag gaa ttc	781	Thr Leu Ala Asn Ile Thr Ser Val Ser Tyr Asp Phe Asp Glu Glu Phe		225	230		235		240	ttc agc cat acg agc gag ctg gcc aag gac ttt att cgg aag ctt ctg	829	Phe Ser His Thr Ser Glu Leu Ala Lys Asp Phe Ile Arg Lys Leu Leu		245	250		255	gtt aaa gag acc ccg aaa ccg ctc aca atc caa gag gct ctc aga cac	877	Val Lys Glu Thr Arg Lys Arg Leu Thr Ile Gln Glu Ala Leu Arg His		260	265		270	ccc tgg atc acg ccg gtg gac aac cag caa gcc atg gtg cga ccg gag	925	Pro Trp Ile Thr Pro Val Asp Asn Gln Gln Ala Met Val Arg Arg Glu		275	280		285	tct gtg gtc aat ctg gag aac ttc agg aag cag tat gtc cgc agg ccg	973	Ser Val Val Asn Leu Glu Asn Phe Arg Lys Gln Tyr Val Arg Arg Arg		290	295		300	tgg aag ctt tcc ttc agc atc gtg tcc ctg tgc aac cac ctc acc cgc	1021	Trp Lys Leu Ser Phe Ser Ile Val Ser Leu Cys Asn His Leu Thr Arg		305	310		315		320	tcg ctg atg aag aag gtg cac ctg agg ccg gat gag gac ctg agg aac	1069	Ser Leu Met Lys Lys Val His Leu Arg Pro Asp Glu Asp Leu Arg Asn		325	330		335																		
	160																																																																																																																
gac ttt ggt ctg gct cac gaa ata gaa gat gga gtt gaa ttt aag aat	589																																																																																																																
Asp Phe Gly Leu Ala His Glu Ile Glu Asp Gly Val Glu Phe Lys Asn																																																																																																																	
165	170		175	att ttt ggg acg ccg gaa ttt gtt gct cca gaa att gtg aac tac gag	637	Ile Phe Gly Thr Pro Glu Phe Val Ala Pro Glu Ile Val Asn Tyr Glu		180	185		190	ccc ctg ggt ctg gag gct gac atg tgg agc ata ggc gtc atc acc tac	685	Pro Leu Gly Leu Glu Ala Asp Met Trp Ser Ile Gly Val Ile Thr Tyr		195	200		205	atc ctc tta agt gga gca tcc cct ttc ctg gga gac acg aag cag gaa	733	Ile Leu Leu Ser Gly Ala Ser Pro Phe Leu Gly Asp Thr Lys Gln Glu		210	215		220	aca ctg gca aat atc aca tca gtg agt tac gac ttt gat gag gaa ttc	781	Thr Leu Ala Asn Ile Thr Ser Val Ser Tyr Asp Phe Asp Glu Glu Phe		225	230		235		240	ttc agc cat acg agc gag ctg gcc aag gac ttt att cgg aag ctt ctg	829	Phe Ser His Thr Ser Glu Leu Ala Lys Asp Phe Ile Arg Lys Leu Leu		245	250		255	gtt aaa gag acc ccg aaa ccg ctc aca atc caa gag gct ctc aga cac	877	Val Lys Glu Thr Arg Lys Arg Leu Thr Ile Gln Glu Ala Leu Arg His		260	265		270	ccc tgg atc acg ccg gtg gac aac cag caa gcc atg gtg cga ccg gag	925	Pro Trp Ile Thr Pro Val Asp Asn Gln Gln Ala Met Val Arg Arg Glu		275	280		285	tct gtg gtc aat ctg gag aac ttc agg aag cag tat gtc cgc agg ccg	973	Ser Val Val Asn Leu Glu Asn Phe Arg Lys Gln Tyr Val Arg Arg Arg		290	295		300	tgg aag ctt tcc ttc agc atc gtg tcc ctg tgc aac cac ctc acc cgc	1021	Trp Lys Leu Ser Phe Ser Ile Val Ser Leu Cys Asn His Leu Thr Arg		305	310		315		320	tcg ctg atg aag aag gtg cac ctg agg ccg gat gag gac ctg agg aac	1069	Ser Leu Met Lys Lys Val His Leu Arg Pro Asp Glu Asp Leu Arg Asn		325	330		335																										
	175																																																																																																																
att ttt ggg acg ccg gaa ttt gtt gct cca gaa att gtg aac tac gag	637																																																																																																																
Ile Phe Gly Thr Pro Glu Phe Val Ala Pro Glu Ile Val Asn Tyr Glu																																																																																																																	
180	185		190	ccc ctg ggt ctg gag gct gac atg tgg agc ata ggc gtc atc acc tac	685	Pro Leu Gly Leu Glu Ala Asp Met Trp Ser Ile Gly Val Ile Thr Tyr		195	200		205	atc ctc tta agt gga gca tcc cct ttc ctg gga gac acg aag cag gaa	733	Ile Leu Leu Ser Gly Ala Ser Pro Phe Leu Gly Asp Thr Lys Gln Glu		210	215		220	aca ctg gca aat atc aca tca gtg agt tac gac ttt gat gag gaa ttc	781	Thr Leu Ala Asn Ile Thr Ser Val Ser Tyr Asp Phe Asp Glu Glu Phe		225	230		235		240	ttc agc cat acg agc gag ctg gcc aag gac ttt att cgg aag ctt ctg	829	Phe Ser His Thr Ser Glu Leu Ala Lys Asp Phe Ile Arg Lys Leu Leu		245	250		255	gtt aaa gag acc ccg aaa ccg ctc aca atc caa gag gct ctc aga cac	877	Val Lys Glu Thr Arg Lys Arg Leu Thr Ile Gln Glu Ala Leu Arg His		260	265		270	ccc tgg atc acg ccg gtg gac aac cag caa gcc atg gtg cga ccg gag	925	Pro Trp Ile Thr Pro Val Asp Asn Gln Gln Ala Met Val Arg Arg Glu		275	280		285	tct gtg gtc aat ctg gag aac ttc agg aag cag tat gtc cgc agg ccg	973	Ser Val Val Asn Leu Glu Asn Phe Arg Lys Gln Tyr Val Arg Arg Arg		290	295		300	tgg aag ctt tcc ttc agc atc gtg tcc ctg tgc aac cac ctc acc cgc	1021	Trp Lys Leu Ser Phe Ser Ile Val Ser Leu Cys Asn His Leu Thr Arg		305	310		315		320	tcg ctg atg aag aag gtg cac ctg agg ccg gat gag gac ctg agg aac	1069	Ser Leu Met Lys Lys Val His Leu Arg Pro Asp Glu Asp Leu Arg Asn		325	330		335																																		
	190																																																																																																																
ccc ctg ggt ctg gag gct gac atg tgg agc ata ggc gtc atc acc tac	685																																																																																																																
Pro Leu Gly Leu Glu Ala Asp Met Trp Ser Ile Gly Val Ile Thr Tyr																																																																																																																	
195	200		205	atc ctc tta agt gga gca tcc cct ttc ctg gga gac acg aag cag gaa	733	Ile Leu Leu Ser Gly Ala Ser Pro Phe Leu Gly Asp Thr Lys Gln Glu		210	215		220	aca ctg gca aat atc aca tca gtg agt tac gac ttt gat gag gaa ttc	781	Thr Leu Ala Asn Ile Thr Ser Val Ser Tyr Asp Phe Asp Glu Glu Phe		225	230		235		240	ttc agc cat acg agc gag ctg gcc aag gac ttt att cgg aag ctt ctg	829	Phe Ser His Thr Ser Glu Leu Ala Lys Asp Phe Ile Arg Lys Leu Leu		245	250		255	gtt aaa gag acc ccg aaa ccg ctc aca atc caa gag gct ctc aga cac	877	Val Lys Glu Thr Arg Lys Arg Leu Thr Ile Gln Glu Ala Leu Arg His		260	265		270	ccc tgg atc acg ccg gtg gac aac cag caa gcc atg gtg cga ccg gag	925	Pro Trp Ile Thr Pro Val Asp Asn Gln Gln Ala Met Val Arg Arg Glu		275	280		285	tct gtg gtc aat ctg gag aac ttc agg aag cag tat gtc cgc agg ccg	973	Ser Val Val Asn Leu Glu Asn Phe Arg Lys Gln Tyr Val Arg Arg Arg		290	295		300	tgg aag ctt tcc ttc agc atc gtg tcc ctg tgc aac cac ctc acc cgc	1021	Trp Lys Leu Ser Phe Ser Ile Val Ser Leu Cys Asn His Leu Thr Arg		305	310		315		320	tcg ctg atg aag aag gtg cac ctg agg ccg gat gag gac ctg agg aac	1069	Ser Leu Met Lys Lys Val His Leu Arg Pro Asp Glu Asp Leu Arg Asn		325	330		335																																										
	205																																																																																																																
atc ctc tta agt gga gca tcc cct ttc ctg gga gac acg aag cag gaa	733																																																																																																																
Ile Leu Leu Ser Gly Ala Ser Pro Phe Leu Gly Asp Thr Lys Gln Glu																																																																																																																	
210	215		220	aca ctg gca aat atc aca tca gtg agt tac gac ttt gat gag gaa ttc	781	Thr Leu Ala Asn Ile Thr Ser Val Ser Tyr Asp Phe Asp Glu Glu Phe		225	230		235		240	ttc agc cat acg agc gag ctg gcc aag gac ttt att cgg aag ctt ctg	829	Phe Ser His Thr Ser Glu Leu Ala Lys Asp Phe Ile Arg Lys Leu Leu		245	250		255	gtt aaa gag acc ccg aaa ccg ctc aca atc caa gag gct ctc aga cac	877	Val Lys Glu Thr Arg Lys Arg Leu Thr Ile Gln Glu Ala Leu Arg His		260	265		270	ccc tgg atc acg ccg gtg gac aac cag caa gcc atg gtg cga ccg gag	925	Pro Trp Ile Thr Pro Val Asp Asn Gln Gln Ala Met Val Arg Arg Glu		275	280		285	tct gtg gtc aat ctg gag aac ttc agg aag cag tat gtc cgc agg ccg	973	Ser Val Val Asn Leu Glu Asn Phe Arg Lys Gln Tyr Val Arg Arg Arg		290	295		300	tgg aag ctt tcc ttc agc atc gtg tcc ctg tgc aac cac ctc acc cgc	1021	Trp Lys Leu Ser Phe Ser Ile Val Ser Leu Cys Asn His Leu Thr Arg		305	310		315		320	tcg ctg atg aag aag gtg cac ctg agg ccg gat gag gac ctg agg aac	1069	Ser Leu Met Lys Lys Val His Leu Arg Pro Asp Glu Asp Leu Arg Asn		325	330		335																																																		
	220																																																																																																																
aca ctg gca aat atc aca tca gtg agt tac gac ttt gat gag gaa ttc	781																																																																																																																
Thr Leu Ala Asn Ile Thr Ser Val Ser Tyr Asp Phe Asp Glu Glu Phe																																																																																																																	
225	230		235		240	ttc agc cat acg agc gag ctg gcc aag gac ttt att cgg aag ctt ctg	829	Phe Ser His Thr Ser Glu Leu Ala Lys Asp Phe Ile Arg Lys Leu Leu		245	250		255	gtt aaa gag acc ccg aaa ccg ctc aca atc caa gag gct ctc aga cac	877	Val Lys Glu Thr Arg Lys Arg Leu Thr Ile Gln Glu Ala Leu Arg His		260	265		270	ccc tgg atc acg ccg gtg gac aac cag caa gcc atg gtg cga ccg gag	925	Pro Trp Ile Thr Pro Val Asp Asn Gln Gln Ala Met Val Arg Arg Glu		275	280		285	tct gtg gtc aat ctg gag aac ttc agg aag cag tat gtc cgc agg ccg	973	Ser Val Val Asn Leu Glu Asn Phe Arg Lys Gln Tyr Val Arg Arg Arg		290	295		300	tgg aag ctt tcc ttc agc atc gtg tcc ctg tgc aac cac ctc acc cgc	1021	Trp Lys Leu Ser Phe Ser Ile Val Ser Leu Cys Asn His Leu Thr Arg		305	310		315		320	tcg ctg atg aag aag gtg cac ctg agg ccg gat gag gac ctg agg aac	1069	Ser Leu Met Lys Lys Val His Leu Arg Pro Asp Glu Asp Leu Arg Asn		325	330		335																																																										
	235		240	ttc agc cat acg agc gag ctg gcc aag gac ttt att cgg aag ctt ctg	829	Phe Ser His Thr Ser Glu Leu Ala Lys Asp Phe Ile Arg Lys Leu Leu		245	250		255	gtt aaa gag acc ccg aaa ccg ctc aca atc caa gag gct ctc aga cac	877	Val Lys Glu Thr Arg Lys Arg Leu Thr Ile Gln Glu Ala Leu Arg His		260	265		270	ccc tgg atc acg ccg gtg gac aac cag caa gcc atg gtg cga ccg gag	925	Pro Trp Ile Thr Pro Val Asp Asn Gln Gln Ala Met Val Arg Arg Glu		275	280		285	tct gtg gtc aat ctg gag aac ttc agg aag cag tat gtc cgc agg ccg	973	Ser Val Val Asn Leu Glu Asn Phe Arg Lys Gln Tyr Val Arg Arg Arg		290	295		300	tgg aag ctt tcc ttc agc atc gtg tcc ctg tgc aac cac ctc acc cgc	1021	Trp Lys Leu Ser Phe Ser Ile Val Ser Leu Cys Asn His Leu Thr Arg		305	310		315		320	tcg ctg atg aag aag gtg cac ctg agg ccg gat gag gac ctg agg aac	1069	Ser Leu Met Lys Lys Val His Leu Arg Pro Asp Glu Asp Leu Arg Asn		325	330		335																																																												
	240																																																																																																																
ttc agc cat acg agc gag ctg gcc aag gac ttt att cgg aag ctt ctg	829																																																																																																																
Phe Ser His Thr Ser Glu Leu Ala Lys Asp Phe Ile Arg Lys Leu Leu																																																																																																																	
245	250		255	gtt aaa gag acc ccg aaa ccg ctc aca atc caa gag gct ctc aga cac	877	Val Lys Glu Thr Arg Lys Arg Leu Thr Ile Gln Glu Ala Leu Arg His		260	265		270	ccc tgg atc acg ccg gtg gac aac cag caa gcc atg gtg cga ccg gag	925	Pro Trp Ile Thr Pro Val Asp Asn Gln Gln Ala Met Val Arg Arg Glu		275	280		285	tct gtg gtc aat ctg gag aac ttc agg aag cag tat gtc cgc agg ccg	973	Ser Val Val Asn Leu Glu Asn Phe Arg Lys Gln Tyr Val Arg Arg Arg		290	295		300	tgg aag ctt tcc ttc agc atc gtg tcc ctg tgc aac cac ctc acc cgc	1021	Trp Lys Leu Ser Phe Ser Ile Val Ser Leu Cys Asn His Leu Thr Arg		305	310		315		320	tcg ctg atg aag aag gtg cac ctg agg ccg gat gag gac ctg agg aac	1069	Ser Leu Met Lys Lys Val His Leu Arg Pro Asp Glu Asp Leu Arg Asn		325	330		335																																																																				
	255																																																																																																																
gtt aaa gag acc ccg aaa ccg ctc aca atc caa gag gct ctc aga cac	877																																																																																																																
Val Lys Glu Thr Arg Lys Arg Leu Thr Ile Gln Glu Ala Leu Arg His																																																																																																																	
260	265		270	ccc tgg atc acg ccg gtg gac aac cag caa gcc atg gtg cga ccg gag	925	Pro Trp Ile Thr Pro Val Asp Asn Gln Gln Ala Met Val Arg Arg Glu		275	280		285	tct gtg gtc aat ctg gag aac ttc agg aag cag tat gtc cgc agg ccg	973	Ser Val Val Asn Leu Glu Asn Phe Arg Lys Gln Tyr Val Arg Arg Arg		290	295		300	tgg aag ctt tcc ttc agc atc gtg tcc ctg tgc aac cac ctc acc cgc	1021	Trp Lys Leu Ser Phe Ser Ile Val Ser Leu Cys Asn His Leu Thr Arg		305	310		315		320	tcg ctg atg aag aag gtg cac ctg agg ccg gat gag gac ctg agg aac	1069	Ser Leu Met Lys Lys Val His Leu Arg Pro Asp Glu Asp Leu Arg Asn		325	330		335																																																																												
	270																																																																																																																
ccc tgg atc acg ccg gtg gac aac cag caa gcc atg gtg cga ccg gag	925																																																																																																																
Pro Trp Ile Thr Pro Val Asp Asn Gln Gln Ala Met Val Arg Arg Glu																																																																																																																	
275	280		285	tct gtg gtc aat ctg gag aac ttc agg aag cag tat gtc cgc agg ccg	973	Ser Val Val Asn Leu Glu Asn Phe Arg Lys Gln Tyr Val Arg Arg Arg		290	295		300	tgg aag ctt tcc ttc agc atc gtg tcc ctg tgc aac cac ctc acc cgc	1021	Trp Lys Leu Ser Phe Ser Ile Val Ser Leu Cys Asn His Leu Thr Arg		305	310		315		320	tcg ctg atg aag aag gtg cac ctg agg ccg gat gag gac ctg agg aac	1069	Ser Leu Met Lys Lys Val His Leu Arg Pro Asp Glu Asp Leu Arg Asn		325	330		335																																																																																				
	285																																																																																																																
tct gtg gtc aat ctg gag aac ttc agg aag cag tat gtc cgc agg ccg	973																																																																																																																
Ser Val Val Asn Leu Glu Asn Phe Arg Lys Gln Tyr Val Arg Arg Arg																																																																																																																	
290	295		300	tgg aag ctt tcc ttc agc atc gtg tcc ctg tgc aac cac ctc acc cgc	1021	Trp Lys Leu Ser Phe Ser Ile Val Ser Leu Cys Asn His Leu Thr Arg		305	310		315		320	tcg ctg atg aag aag gtg cac ctg agg ccg gat gag gac ctg agg aac	1069	Ser Leu Met Lys Lys Val His Leu Arg Pro Asp Glu Asp Leu Arg Asn		325	330		335																																																																																												
	300																																																																																																																
tgg aag ctt tcc ttc agc atc gtg tcc ctg tgc aac cac ctc acc cgc	1021																																																																																																																
Trp Lys Leu Ser Phe Ser Ile Val Ser Leu Cys Asn His Leu Thr Arg																																																																																																																	
305	310		315		320	tcg ctg atg aag aag gtg cac ctg agg ccg gat gag gac ctg agg aac	1069	Ser Leu Met Lys Lys Val His Leu Arg Pro Asp Glu Asp Leu Arg Asn		325	330		335																																																																																																				
	315		320	tcg ctg atg aag aag gtg cac ctg agg ccg gat gag gac ctg agg aac	1069	Ser Leu Met Lys Lys Val His Leu Arg Pro Asp Glu Asp Leu Arg Asn		325	330		335																																																																																																						
	320																																																																																																																
tcg ctg atg aag aag gtg cac ctg agg ccg gat gag gac ctg agg aac	1069																																																																																																																
Ser Leu Met Lys Lys Val His Leu Arg Pro Asp Glu Asp Leu Arg Asn																																																																																																																	
325	330		335																																																																																																														
	335																																																																																																																

tgt gag agt gac act gag gag gac atc gcc agg agg aaa gcc ctc cac 1117
 Cys Glu Ser Asp Thr Glu Glu Asp Ile Ala Arg Arg Lys Ala Leu His
 340 345 350 350

cca cg agg agg agc acc tcc taactggctt gacactgagtt ggccggcagg 1171
 Pro Arg Arg Arg Ser Ser Thr Ser
 355 360

gagggtttggg cccagcgggg ctcccttctg tgcagacttt tggaccaggc tcagcaccag 1231
 caccggggcg tcctgagcac tttgcagag agatggggcc aaggaattca gaagagctt 1291
 caggcaagcc aggagaccct gggagctgtg gctgttcttctt gtggggaggc ctccagcatt 1351
 cccaaagctc ttaattctcc ataaaatggg ctttcttctg tctgcattcc tcagactctg 1411
 ggggtggagt gtggacttag gaaaacaata taaaggacat cctcatcata acgggggtgaa 1471
 ggtcagagta aggacgcctt ctccacaggc tgagggggtt cagaaccaggc ctggccaaaa 1531
 attacaccag agagacagag tcctcccat tgggaaacagg gtgattgagg aaaaatgtaaacc 1591
 ttgggtgtga gggaccaatc ctgtgaccc tcagaaccat ggaagccagg acgtcaggct 1651
 gaccaacacc tcagacccctc tgaaggcagcc cattgctggc ccgcctatgtt gtaatttgc 1711
 tcatttttat taaacttctg gtttacactga a 1742

<210> 2
 <211> 360
 <212> PRT
 <213> Human

<400> 2
 Met Glu Pro Phe Lys Gln Gln Lys Val Glu Asp Phe Tyr Asp Ile Gly
 1 5 10 15

Glu Glu Leu Gly Ser Gly Gln Phe Ala Ile Val Lys Lys Cys Arg Glu
 20 25 30

Lys Ser Thr Gly Leu Glu Tyr Ala Ala Lys Phe Ile Lys Lys Arg Gln
 35 40 45

Ser Arg Ala Ser Arg Arg Gly Val Ser Arg Glu Glu Ile Glu Arg Glu
 50 55 60

Val Ser Ile Leu Arg Gln Val Leu His His Asn Val Ile Thr Leu His
 65 70 75 80

Asp Val Tyr Glu Asn Arg Thr Asp Val Val His Ile Leu Glu Leu Val
 85 90 95

Ser Gly Gly Glu Leu Phe Asp Phe Leu Ala Gln Lys Glu Ser Leu Ser
 100 105 110

Glu Glu Glu Ala Thr Ser Phe Ile Lys Gln Ile Leu Asp Gly Val Asn
 115 120 125

Tyr Leu His Thr Lys Lys Ile Ala His Phe Asp Leu Lys Pro Glu Asn
 130 135 140
 Ile Met Leu Leu Asp Lys Asn Ile Pro Ile Pro His Ile Lys Leu Ile
 145 150 155 160
 Asp Phe Gly Leu Ala His Glu Ile Glu Asp Gly Val Glu Phe Lys Asn
 165 170 175
 Ile Phe Gly Thr Pro Glu Phe Val Ala Pro Glu Ile Val Asn Tyr Glu
 180 185 190
 Pro Leu Gly Leu Glu Ala Asp Met Trp Ser Ile Gly Val Ile Thr Tyr
 195 200 205
 Ile Leu Leu Ser Gly Ala Ser Pro Phe Leu Gly Asp Thr Lys Gln Glu
 210 215 220
 Thr Leu Ala Asn Ile Thr Ser Val Ser Tyr Asp Phe Asp Glu Glu Phe
 225 230 235 240
 Phe Ser His Thr Ser Glu Leu Ala Lys Asp Phe Ile Arg Lys Leu Leu
 245 250 255
 Val Lys Glu Thr Arg Lys Arg Leu Thr Ile Gln Glu Ala Leu Arg His
 260 265 270
 Pro Trp Ile Thr Pro Val Asp Asn Gln Gln Ala Met Val Arg Arg Glu
 275 280 285
 Ser Val Val Asn Leu Glu Asn Phe Arg Lys Gln Tyr Val Arg Arg Arg
 290 295 300
 Trp Lys Leu Ser Phe Ser Ile Val Ser Leu Cys Asn His Leu Thr Arg
 305 310 315 320
 Ser Leu Met Lys Lys Val His Leu Arg Pro Asp Glu Asp Leu Arg Asn
 325 330 335
 Cys Glu Ser Asp Thr Glu Glu Asp Ile Ala Arg Arg Lys Ala Leu His
 340 345 350
 Pro Arg Arg Arg Ser Ser Thr Ser
 355 360

 <210> 3
 <211> 263
 <212> PRT
 <213> Human

 <400> 3
 Tyr Asp Thr Gly Glu Glu Leu Gly Ser Gly Gln Phe Ala Val Val Lys
 1 5 10 15
 Lys Cys Arg Glu Lys Ser Thr Gly Leu Gln Tyr Ala Ala Lys Phe Ile
 20 25 30

09/220-211/67462
Lys Lys Arg Arg Thr Lys Ser Ser Arg Arg Gly Val Ser Arg Glu Asp
35 40 45
Ile Glu Arg Glu Val Ser Ile Leu Lys Glu Ile Gln His Pro Asn Val
50 55 60
Ile Thr Leu His Glu Val Tyr Glu Asn Lys Thr Asp Val Ile Leu Ile
65 70 75 80
Leu Glu Leu Val Ala Gly Gly Glu Leu Phe Asp Phe Leu Ala Glu Lys
85 90 95
Glu Ser Leu Thr Glu Glu Ala Thr Glu Phe Leu Lys Gln Ile Leu
100 105 110
Asn Gly Val Tyr Tyr Leu His Ser Leu Gln Ile Ala His Phe Asp Leu
115 120 125
Lys Pro Glu Asn Ile Met Leu Leu Asp Arg Asn Val Pro Lys Pro Arg
130 135 140
Ile Lys Ile Ile Asp Phe Gly Leu Ala His Lys Ile Asp Phe Gly Asn
145 150 155 160
Glu Phe Lys Asn Ile Phe Gly Thr Pro Glu Phe Val Ala Pro Glu Ile
165 170 175
Val Asn Tyr Glu Pro Leu Gly Leu Glu Ala Asp Met Trp Ser Ile Gly
180 185 190
Val Ile Thr Tyr Ile Leu Leu Ser Gly Ala Ser Pro Phe Leu Gly Asp
195 200 205
Thr Lys Gln Glu Thr Leu Ala Asn Val Ser Ala Val Asn Tyr Glu Phe
210 215 220
Glu Asp Glu Tyr Phe Ser Asn Thr Ser Ala Leu Ala Lys Asp Phe Ile
225 230 235 240
Arg Arg Leu Leu Val Lys Asp Pro Lys Lys Arg Met Thr Ile Gln Asp
245 250 255
Ser Leu Gln His Pro Trp Ile
260

<210> 4
<211> 263
<212> PRT
<213> Human

<400> 4
Tyr Glu Met Gly Glu Glu Leu Gly Ser Gly Gln Phe Ala Ile Val Arg
1 5 10 15
Lys Cys Arg Gln Lys Gly Thr Gly Lys Glu Tyr Ala Ala Lys Phe Ile
20 25 30

Lys Lys Arg Arg Leu Ser Ser Ser Arg Arg Gly Val Ser Arg Glu Glu
 35 40 45
 Ile Glu Arg Glu Val Asn Ile Leu Arg Glu Ile Arg His Pro Asn Ile
 50 55 60
 Ile Thr Leu His Asp Ile Phe Glu Asn Lys Thr Asp Val Val Leu Ile
 65 70 75 80
 Leu Glu Leu Val Ser Gly Gly Glu Leu Phe Asp Phe Leu Ala Glu Lys
 85 90 95
 Glu Ser Leu Thr Glu Asp Glu Ala Thr Gln Phe Leu Lys Gln Ile Leu
 100 105 110
 Asp Gly Val His Tyr Leu His Ser Lys Arg Ile Ala His Phe Asp Leu
 115 120 125
 Lys Pro Glu Asn Ile Met Leu Leu Asp Lys Asn Val Pro Asn Pro Arg
 130 135 140
 Ile Lys Leu Ile Asp Phe Gly Ile Ala His Lys Ile Glu Ala Gly Asn
 145 150 155 160
 Glu Phe Lys Asn Ile Phe Gly Thr Pro Glu Phe Val Ala Pro Glu Ile
 165 170 175
 Val Asn Tyr Glu Pro Leu Gly Leu Glu Ala Asp Met Trp Ser Ile Gly
 180 185 190
 Val Ile Thr Tyr Ile Leu Leu Ser Gly Ala Ser Pro Phe Leu Gly Glu
 195 200 205
 Thr Lys Gln Glu Thr Leu Thr Asn Ile Ser Ala Val Asn Tyr Asp Phe
 210 215 220
 Asp Glu Glu Tyr Phe Ser Asn Thr Ser Glu Leu Ala Lys Asp Phe Ile
 225 230 235 240
 Arg Arg Leu Leu Val Lys Asp Pro Lys Arg Arg Met Thr Ile Ala Gln
 245 250 255
 Ser Leu Glu His Ser Trp Ile
 260

<210> 5
 <211> 261
 <212> PRT
 <213> Human

<400> 5
 Leu Cys Pro Gly Arg Glu Leu Gly Arg Gly Lys Phe Ala Val Val Arg
 1 5 10 15

Lys Cys Ile Lys Lys Asp Ser Gly Lys Glu Phe Ala Ala Lys Phe Met
 20 25 30

Arg Lys Arg Arg Lys Gly Gln Asp Cys Arg Met Glu Ile Ile His Glu
 35 40 45
 Ile Ala Val Leu Glu Leu Ala Gln Asp Asn Pro Trp Val Ile Asn Leu
 50 55 60
 His Glu Val Tyr Glu Thr Ala Ser Glu Met Ile Leu Val Leu Glu Tyr
 65 70 75 80
 Ala Ala Gly Glu Ile Phe Asp Gln Cys Val Ala Asp Arg Glu Glu
 85 90 95
 Ala Phe Lys Glu Lys Asp Val Gln Arg Leu Met Arg Gln Ile Leu Glu
 100 105 110
 Gly Val His Phe Leu His Thr Arg Asp Val Val His Leu Asp Leu Lys
 115 120 125
 Pro Gln Asn Ile Leu Leu Thr Ser Glu Ser Pro Leu Gly Asp Ile Lys
 130 135 140
 Ile Val Asp Phe Gly Leu Ser Arg Ile Leu Lys Asn Ser Glu Glu Leu
 145 150 155 160
 Arg Glu Ile Met Gly Thr Pro Glu Tyr Val Ala Pro Glu Ile Leu Ser
 165 170 175
 Tyr Asp Pro Ile Ser Met Ala Thr Asp Met Trp Ser Ile Gly Val Leu
 180 185 190
 Thr Tyr Val Met Leu Thr Gly Ile Ser Pro Phe Leu Gly Asn Asp Lys
 195 200 205
 Gln Glu Thr Phe Leu Asn Ile Ser Gln Met Asn Leu Ser Tyr Ser Glu
 210 215 220
 Glu Glu Phe Asp Val Leu Ser Glu Ser Ala Val Asp Phe Ile Arg Thr
 225 230 235 240
 Leu Leu Val Lys Lys Pro Glu Asp Arg Ala Thr Ala Glu Glu Cys Leu
 245 250 255
 Lys His Pro Trp Leu
 260

<210> 6
 <211> 261
 <212> PRT
 <213> Human

<400> 6
 Ile Leu Thr Ser Lys Glu Leu Gly Arg Gly Lys Phe Ala Val Val Arg
 1 5 10 15
 Gln Cys Ile Ser Lys Ser Thr Gly Gln Glu Tyr Ala Ala Lys Phe Leu
 20 25 30

09279276727207

Lys Lys Arg Arg Arg Gly Gln Asp Cys Arg Ala Glu Ile Leu His Glu
35 40 45

Ile Ala Val Leu Glu Leu Ala Lys Ser Cys Pro Arg Val Ile Asn Leu
50 55 60

His Glu Val Tyr Glu Asn Thr Ser Glu Ile Ile Leu Ile Leu Glu Tyr
65 70 75 80

Ala Ala Gly Gly Glu Ile Phe Ser Leu Cys Leu Pro Glu Leu Ala Glu
85 90 95

Met Val Ser Glu Asn Asp Val Ile Arg Leu Ile Lys Gln Ile Leu Glu
100 105 110

Gly Val Tyr Tyr Leu His Gln Asn Asn Ile Val His Leu Asp Leu Lys
115 120 125

Pro Gln Asn Ile Leu Leu Ser Ser Ile Tyr Pro Leu Gly Asp Ile Lys
130 135 140

Ile Val Asp Phe Gly Met Ser Arg Lys Ile Gly His Ala Cys Glu Leu
145 150 155 160

Arg Glu Ile Met Gly Thr Pro Glu Tyr Leu Ala Pro Glu Ile Leu Asn
165 170 175

Tyr Asp Pro Ile Thr Thr Ala Thr Asp Met Trp Asn Ile Gly Ile Ile
180 185 190

Ala Tyr Met Leu Leu Thr His Thr Ser Pro Phe Val Gly Glu Asp Asn
195 200 205

Gln Glu Thr Tyr Leu Asn Ile Ser Gln Val Asn Val Asp Tyr Ser Glu
210 215 220

Glu Thr Phe Ser Ser Val Ser Gln Leu Ala Thr Asp Phe Ile Gln Ser
225 230 235 240

Leu Leu Val Lys Asn Pro Glu Lys Arg Pro Thr Ala Glu Ile Cys Leu
245 250 255

Ser His Ser Trp Leu
260

<210> 7
<211> 29
<212> PRT
<213> Human

<400> 7
Asn Met Glu Lys Phe Lys Lys Phe Ala Ala Arg Lys Lys Trp Lys Gln
1 5 10 15

Ser Val Arg Leu Ile Ser Leu Cys Gln Arg Leu Ser Arg
20 25

<210> 8
<211> 29
<212> PRT
<213> Human

<400> 8
Ser Lys Asp Arg Met Lys Lys Tyr Met Ala Arg Arg Lys Trp Gln Lys
1 5 10 15
Thr Gly His Ala Val Arg Ala Ile Gly Arg Leu Ser Ser
20 25

<210> 9
<211> 29
<212> PRT
<213> Human

<400> 9
Thr Val Asp Cys Leu Lys Lys Leu Asn Ala Arg Arg Lys Leu Lys Gly
1 5 10 15
Ala Ile Leu Thr Thr Met Leu Ala Thr Arg Asn Phe Ser
20 25

<210> 10
<211> 28
<212> PRT
<213> Human

<400> 10
Val Ser Glu Gln Ile Lys Lys Asn Phe Ala Lys Ser Lys Trp Lys Gln
1 5 10 15
Ala Phe Asn Ala Thr Ala Val Val Arg His Met Arg
20 25

<210> 11
<211> 32
<212> PRT
<213> Human

<400> 11
Met Asp Thr Ala Gln Lys Lys Leu Glu Phe Asn Ala Arg Arg Lys
1 5 10 15
Leu Lys Ala Ala Val Lys Ala Val Val Ala Ser Ser Arg Leu Gly Ser
20 25 30

<210> 12
<211> 28

<212> PRT
<213> Human

<400> 12
Gly Glu Asp Ser Gly Arg Lys Pro Glu Arg Arg Arg Leu Lys Thr Thr
1 5 10 15

Arg Leu Lys Glu Tyr Thr Ile Lys Ser His Ser Ser
20 25

<210> 13
<211> 20
<212> DNA
<213> Human

<400> 13
ggccggatga ggacctgagg 20

<210> 14
<211> 21
<212> DNA
<213> Human

<400> 14
tccacatccc accccagact c 21

10/220 34/67460

09/719748

JEP Rec'd PCT/PTO 15 DEC 2000
PCT/US99/13411

WO 99/66030

SEQUENCE LISTING

<110> KIMCHI, Adi
 MCINNIS A., Patricia
 YEDA RESEARCH AND DEVELOPMENT COMPANY LTD.

<120> DAP-KINASE RELATED PROTEIN

<130> KIMCHI2A

<140> 00
 <141> 1999-06-15

<150> 60/089,294
 <151> 1998-06-15

<160> 14

<170> PatentIn Ver. 2.0

<210> 1
 <211> 1742
 <212> DNA
 <213> Human

<220>
 <221> CDS
 <222> (62)..(1141)

<400> 1

gaccgcggca gctcagcctc cccgcgattg tatgttccag gcctcaatga ggagtccaaa 60

c atg gag cca ttc aag cag cag aag gtg gag gac ttt tat gac atc gga 109
 Met Glu Pro Phe Lys Gln Gln Lys Val Glu Asp Phe Tyr Asp Ile Gly
 1 5 10 15

gag gag ctg ggg agt ggc cag ttt gcc atc gtg aag aag tgc cgg gag 157
 Glu Glu Leu Gly Ser Gly Gln Phe Ala Ile Val Lys Lys Cys Arg Glu
 20 25 30

aag agc acg ggg ctt gag tat gca gcc aag ttc atc aag aag cgg cag 205
 Lys Ser Thr Gly Leu Glu Tyr Ala Ala Lys Phe Ile Lys Lys Arg Gln
 35 40 45

agc cgg gcg agc cgg cgc ggt gtg agc cgg gag gag atc gag cgg gag 253
 Ser Arg Ala Ser Arg Arg Gly Val Ser Arg Glu Glu Ile Glu Arg Glu
 50 55 60

gtg agc atc ctg cgg cag gtg ctg cac cac aat gtc atc acg ctg cac 301
 Val Ser Ile Leu Arg Gln Val Leu His His Asn Val Ile Thr Leu His
 65 70 75 80

gac gtc tat gag aac cgc acc gac gtg gtg cac atc ctt gag cta gtg 349
 Asp Val Tyr Glu Asn Arg Thr Asp Val Val His Ile Leu Glu Leu Val
 85 90 95

tct gga gga gag ctc ttc gat ttc ctg gcc cag aag gag tca ctg agt 397
 .Ser Gly Gly Glu Leu Phe Asp Phe Leu Ala Gln Lys Glu Ser Leu Ser
 100 105 110

gag gag gag gcc acc agc ttc att aag cag atc ctg gat ggg gtg aac 445
 Glu Glu Glu Ala Thr Ser Phe Ile Lys Gln Ile Leu Asp Gly Val Asn
 115 120 125

tac ctt cac aca aag aaa att gct cac ttt gat ctc aag cca gaa aac 493
 Tyr Leu His Thr Lys Lys Ile Ala His Phe Asp Leu Lys Pro Glu Asn
 130 135 140

att atg ttg tta gac aag aat att ccc att cca cac atc aag ctg att 541
 Ile Met Leu Leu Asp Lys Asn Ile Pro His Ile Lys Leu Ile
 145 150 155 160

gac ttt ggt ctg gct cac gaa ata gaa gat gga gtt gaa ttt aag aat 589
 Asp Phe Gly Leu Ala His Glu Ile Glu Asp Gly Val Glu Phe Lys Asn
 165 170 175

att ttt ggg acg ccg gaa ttt gtt gct cca gaa att gtg aac tac gag 637
 Ile Phe Gly Thr Pro Glu Phe Val Ala Pro Glu Ile Val Asn Tyr Glu
 180 185 190

ccc ctg ggt ctg gag gct gac atg tgg agc ata ggc gtc atc acc tac 685
 Pro Leu Gly Leu Ala Asp Met Trp Ser Ile Gly Val Ile Thr Tyr
 195 200 205

atc ctc tta agt gga gca tcc cct ttc ctg gga gac acg aag cag gaa 733
 Ile Leu Leu Ser Gly Ala Ser Pro Phe Leu Gly Asp Thr Lys Gln Glu
 210 215 220

aca ctg gca aat atc aca tca gtg agt tac gac ttt gat gag gaa ttc 781
 Thr Leu Ala Asn Ile Thr Ser Val Ser Tyr Asp Phe Asp Glu Glu Phe
 225 230 235 240

ttc agc cat acg agc gag ctg gcc aag gac ttt att cgg aag ctt ctg 829
 Phe Ser His Thr Ser Glu Leu Ala Lys Asp Phe Ile Arg Lys Leu Leu
 245 250 255

70/2220-34/267260

gtt aaa gag acc cg^g aaa cg^g ctc aca atc caa gag gct ctc aga cac 877
 Val Lys Glu Thr Arg Lys Arg Leu Thr Ile Gln Glu Ala Leu Arg His
 260 265 270

ccc tgg atc acg cg^g gtg gac aac cag caa gcc atg gtg cga cg^g gag 925
 Pro Trp Ile Thr Pro Val Asp Asn Gln Gln Ala Met Val Arg Arg Glu
 275 280 285

tct gtg gtc aat ctg gag aac ttc agg aag cag tat gtc cgc agg cg^g 973
 Ser Val Val Asn Leu Glu Asn Phe Arg Lys Gln Tyr Val Arg Arg Arg
 290 295 300

tgg aag ctt tcc ttc agc atc gtg tcc ctg tgc aac cac ctc acc cgc 1021
 Trp Lys Leu Ser Phe Ser Ile Val Ser Leu Cys Asn His Leu Thr Arg
 305 310 315 320

tcg ctg atg aag aag gtg cac ctg agg cg^g gat gag gac ctg agg aac 1069
 Ser Leu Met Lys Lys Val His Leu Arg Pro Asp Glu Asp Leu Arg Asn
 325 330 335

tgt gag agt gac act gag gac atc gcc agg agg aaa gcc ctc cac 1117
 Cys Glu Ser Asp Thr Glu Glu Asp Ile Ala Arg Arg Lys Ala Leu His
 340 345 350

cca cg^g agg agg agc acc tcc taactggcct gacctgcagt ggccggcagg 1171
 Pro Arg Arg Arg Ser Ser Thr Ser
 355 360

gagggtttggg cccagcg^{gggg} ctcccttctg tgcagacttt tggaccgc t^{ca}gaccagg 1231

cacccg^{gggg}cg tcttgagcac tt^tgcaagag agatggccca aaggaattca gaagagcttg 1291

caggcaagcc aggagaccct gggagctgtg gctgtcttct gtggaggagg ctccagcatt 1351

cccaaaagctc ttaattctcc ataaaatggg ct^tttctctg tctgcccattc t^{ca}gagtc^tctg 1411

gggtgggagt gtggacttag gaaaacaata taaaggacat cctcatc^tacgggggtgaa 1471

ggtcagagta aggcagcc^tttt c^ttcacaggc tgagggggtt cagaaccagg ctggccaaaa 1531

attacaccag agagacagag t^tcttccccat tggaaacagg gtgattgagg aaagtgaacc 1591

ttgggtgtga gggccaatc ctgtgac^ttc ccagaaccat ggaaggccagg acgtcagg^tct 1651

gaccaacacc tcagaccc^ttc tgaagcagcc cattgctggc cgc^tccatgtt gtaatttgc 1711

tcatttttat taaaacttctg gtttacactga a 1742

<210> 2
<211> 360
<212> PRT
<213> Human

<400> 2
Met Glu Pro Phe Lys Gln Gln Lys Val Glu Asp Phe Tyr Asp Ile Gly
1 5 10 15

Glu Glu Leu Gly Ser Gly Gln Phe Ala Ile Val Lys Lys Cys Arg Glu
20 25 30

Lys Ser Thr Gly Leu Glu Tyr Ala Ala Lys Phe Ile Lys Lys Arg Gln
35 40 45

Ser Arg Ala Ser Arg Arg Gly Val Ser Arg Glu Glu Ile Glu Arg Glu
50 55 60

Val Ser Ile Leu Arg Gln Val Leu His His Asn Val Ile Thr Leu His
65 70 75 80

Asp Val Tyr Glu Asn Arg Thr Asp Val Val His Ile Leu Glu Leu Val
85 90 95

Ser Gly Gly Glu Leu Phe Asp Phe Leu Ala Gln Lys Glu Ser Leu Ser
100 105 110

Glu Glu Glu Ala Thr Ser Phe Ile Lys Gln Ile Leu Asp Gly Val Asn
115 120 125

Tyr Leu His Thr Lys Lys Ile Ala His Phe Asp Leu Lys Pro Glu Asn
130 135 140

Ile Met Leu Leu Asp Lys Asn Ile Pro Ile Pro His Ile Lys Leu Ile
145 150 155 160

Asp Phe Gly Leu Ala His Glu Ile Glu Asp Gly Val Glu Phe Lys Asn
165 170 175

Ile Phe Gly Thr Pro Glu Phe Val Ala Pro Glu Ile Val Asn Tyr Glu
180 185 190

Pro Leu Gly Leu Glu Ala Asp Met Trp Ser Ile Gly Val Ile Thr Tyr
195 200 205

Ile Leu Leu Ser Gly Ala Ser Pro Phe Leu Gly Asp Thr Lys Gln Glu
210 215 220

Thr Leu Ala Asn Ile Thr Ser Val Ser Tyr Asp Phe Asp Glu Glu Phe
 225 230 235 240

Phe Ser His Thr Ser Glu Leu Ala Lys Asp Phe Ile Arg Ly Leu Leu
 245 250 255

Val Lys Glu Thr Arg Lys Arg Leu Thr Ile Gln Glu Ala Le Arg His
 260 265 27

Pro Trp Ile Thr Pro Val Asp Asn Gln Gln Ala Met Val Ar Arg Glu
 275 280 285

Ser Val Val Asn Leu Glu Asn Phe Arg Lys Gln Tyr Val Ar Arg Arg
 290 295 300

Trp Lys Leu Ser Phe Ser Ile Val Ser Leu Cys Asn His Le Thr Arg
 305 310 315 320

Ser Leu Met Lys Lys Val His Leu Arg Pro Asp Glu Asp Le Arg Asn
 325 330 335

Cys Glu Ser Asp Thr Glu Glu Asp Ile Ala Arg Arg Lys Al Leu His
 340 345 35

Pro Arg Arg Arg Ser Ser Thr Ser
 355 360

<210> 3

<211> 263

<212> PRT

<213> Human

<400> 3

Tyr Asp Thr Gly Glu Glu Leu Gly Ser Gly Gln Phe Ala V Val Lys
 1 5 10 15

Lys Cys Arg Glu Lys Ser Thr Gly Leu Gln Tyr Ala Ala L Phe Ile
 20 25

Lys Lys Arg Arg Thr Lys Ser Ser Arg Arg Gly Val Ser A : Glu Asp
 35 40 45

Ile Glu Arg Glu Val Ser Ile Leu Lys Glu Ile Gln His P Asn Val
 50 55 60

Ile Thr Leu His Glu Val Tyr Glu Asn Lys Thr Asp Val I : Leu Ile

65	70	75	80
Leu Glu Leu Val Ala Gly Gly Glu Leu Phe Asp Phe Leu Al Glu Lys			
85	90	95	
Glu Ser Leu Thr Glu Glu Ala Thr Glu Phe Leu Lys Gl Ile Leu			
100	105	110	
Asn Gly Val Tyr Tyr Leu His Ser Leu Gln Ile Ala His P Asp Leu			
115	120	125	
Lys Pro Glu Asn Ile Met Leu Leu Asp Arg Asn Val Pro L Pro Arg			
130	135	140	
Ile Lys Ile Ile Asp Phe Gly Leu Ala His Lys Ile Asp P Gly Asn			
145	150	155	160
Glu Phe Lys Asn Ile Phe Gly Thr Pro Glu Phe Val Ala P Glu Ile			
165	170	175	
Val Asn Tyr Glu Pro Leu Gly Leu Glu Ala Asp Met Trp S Ile Gly			
180	185	190	
Val Ile Thr Tyr Ile Leu Leu Ser Gly Ala Ser Pro Phe L Ile Gly Asp			
195	200	205	
Thr Lys Gln Glu Thr Leu Ala Asn Val Ser Ala Val Asn Ile Glu Phe			
210	215	220	
Glu Asp Glu Tyr Phe Ser Asn Thr Ser Ala Leu Ala Lys Ile Phe Ile			
225	230	235	240
Arg Arg Leu Leu Val Lys Asp Pro Lys Lys Arg Met Thr Gln Asp			
245	250	255	
Ser Leu Gln His Pro Trp Ile			
260			

<210> 4
 <211> 263
 <212> PRT
 <213> Human

<400> 4
 Tyr Glu Met Gly Glu Glu Leu Gly Ser Gly Gln Phe Ala Le Val Arg
 1 5 10 15

Lys Cys Arg Gln Lys Gly Thr Gly Lys Glu Tyr Ala Ala Lys Phe Ile
20 25 30

Lys Lys Arg Arg Leu Ser Ser Ser Arg Arg Gly Val Ser Arg Glu Glu
35 40 45

Ile Glu Arg Glu Val Asn Ile Leu Arg Glu Ile Arg His Pro Asn Ile
50 55 60

Ile Thr Leu His Asp Ile Phe Glu Asn Lys Thr Asp Val Val Leu Ile
65 70 75 80

Leu Glu Leu Val Ser Gly Gly Glu Leu Phe Asp Phe Leu Ala Glu Lys
85 90 95

Glu Ser Leu Thr Glu Asp Glu Ala Thr Gln Phe Leu Lys Gln Ile Leu
100 105 110

Asp Gly Val His Tyr Leu His Ser Lys Arg Ile Ala His Phe Asp Leu
115 120 125

Lys Pro Glu Asn Ile Met Leu Leu Asn Lys Asn Val Pro Asn Pro Arg
130 135 140

Ile Lys Leu Ile Asp Phe Gly Ile Ala His Lys Ile Glu Ala Gly Asn
145 150 155 160

Glu Phe Lys Asn Ile Phe Gly Thr Pro Glu Phe Val Ala Pro Glu Ile
165 170 175

Val Asn Tyr Glu Pro Leu Gly Leu Glu Ala Asp Met Trp Ser Ile Gly
180 185 190

Val Ile Thr Tyr Ile Leu Leu Ser Gly Ala Ser Pro Phe Leu Gly Glu
195 200 205

Thr Lys Gln Glu Thr Leu Thr Asn Ile Ser Ala Val Asn Tyr Asp Phe
210 215 220

Asp Glu Glu Tyr Phe Ser Asn Thr Ser Glu Leu Ala Lys Asp Phe Ile
225 230 235 240

Arg Arg Leu Leu Val Lys Asp Pro Lys Arg Arg Met Thr Ile Ala Gln
245 250 255

Ser Leu Glu His Ser Trp Ile
260

<210> 5
<211> 261
<212> PRT
<213> Human

<400> 5
Leu Cys Pro Gly Arg Glu Leu Gly Arg Gly Lys Phe Ala Val Val Arg
1 5 10 15

Lys Cys Ile Lys Lys Asp Ser Gly Lys Glu Phe Ala Ala Lys Phe Met
20 25 30

Arg Lys Arg Arg Lys Gly Gln Asp Cys Arg Met Glu Ile Ile His Glu
35 40 45

Ile Ala Val Leu Glu Leu Ala Gln Asp Asn Pro Trp Val Ile Asn Leu
50 55 60

*His Glu Val Tyr Glu Thr Ala Ser Glu Met Ile Leu Val Leu Glu Tyr
65 70 75 80

Ala Ala Gly Gly Glu Ile Phe Asp Gln Cys Val Ala Asp Arg Glu Glu
85 90 95

Ala Phe Lys Glu Lys Asp Val Gln Arg Leu Met Arg Gln Ile Leu Glu
100 105 110

Gly Val His Phe Leu His Thr Arg Asp Val Val His Leu Asp Leu Lys
115 120 125

Pro Gln Asn Ile Leu Leu Thr Ser Glu Ser Pro Leu Gly Asp Ile Lys
130 135 140

Ile Val Asp Phe Gly Leu Ser Arg Ile Leu Lys Asn Ser Glu Glu Leu
145 150 155 160

Arg Glu Ile Met Gly Thr Pro Glu Tyr Val Ala Pro Glu Ile Leu Ser
165 170 175

Tyr Asp Pro Ile Ser Met Ala Thr Asp Met Trp Ser Ile Gly Val Leu
180 185 190

Thr Tyr Val Met Leu Thr Gly Ile Ser Pro Phe Leu Gly Asn Asp Lys
195 200 205

Gln Glu Thr Phe Leu Asn Ile Ser Gln Met Asn Leu Ser Tyr Ser Glu
210 215 220

Glu Glu Phe Asp Val Leu Ser Glu Ser Ala Val Asp Phe Ile Arg Thr
225 230 235 240

Leu Leu Val Lys Lys Pro Glu Asp Arg Ala Thr Ala Glu Glu Cys Leu
245 250 255

Lys His Pro Trp Leu
260

<210> 6

<211> 261

<212> PRT

<213> Human

<400> 6

Ile Leu Thr Ser Lys Glu Leu Gly Arg Gly Lys Phe Ala Val Val Arg
1 5 10 15

Gln Cys Ile Ser Lys Ser Thr Gly Gln Glu Tyr Ala Ala Lys Phe Leu
20 25 30

Lys Lys Arg Arg Arg Gly Gln Asp Cys Arg Ala Glu Ile Leu His Glu
35 40 45

Ile Ala Val Leu Glu Leu Ala Lys Ser Cys Pro Arg Val Ile Asn Leu
50 55 60

His Glu Val Tyr Glu Asn Thr Ser Glu Ile Ile Leu Ile Leu Glu Tyr
65 70 75 80

Ala Ala Gly Gly Glu Ile Phe Ser Leu Cys Leu Pro Glu Leu Ala Glu
85 90 95

Met Val Ser Glu Asn Asp Val Ile Arg Leu Ile Lys Gln Ile Leu Glu
100 105 110

Gly Val Tyr Tyr Leu His Gln Asn Asn Ile Val His Leu Asp Leu Lys
115 120 125

Pro Gln Asn Ile Leu Leu Ser Ser Ile Tyr Pro Leu Gly Asp Ile Lys
130 135 140

Ile Val Asp Phe Gly Met Ser Arg Lys Ile Gly His Ala Cys Glu Leu
145 150 155 160

Arg Glu Ile Met Gly Thr Pro Glu Tyr Leu Ala Pro Glu Ile Leu Asn

165

170

175

Tyr Asp Pro Ile Thr Thr Ala Thr Asp Met Trp Asn Ile Gly Ile Ile
180 185 190

Ala Tyr Met Leu Leu Thr His Thr Ser Pro Phe Val Gly Glu Asp Asn
195 200 205

Gln Glu Thr Tyr Leu Asn Ile Ser Gln Val Asn Val Asp Tyr Ser Glu
210 215 220

Glu Thr Phe Ser Ser Val Ser Gln Leu Ala Thr Asp Phe Ile Gln Ser
225 230 235 240

Leu Leu Val Lys Asn Pro Glu Lys Arg Pro Thr Ala Glu Ile Cys Leu
245 250 255

Ser His Ser Trp Leu
260

<210> 7

<211> 29

<212> PRT

<213> Human

<400> 7

Asn Met Glu Lys Phe Lys Lys Phe Ala Ala Arg Lys Lys Trp Lys Gln
1 5 10 15

Ser Val Arg Leu Ile Ser Leu Cys Gln Arg Leu Ser Arg
20 25

<210> 8

<211> 29

<212> PRT

<213> Human

<400> 8

Asn Leu Glu Asn Phe Arg Lys Gln Tyr Val Arg Arg Arg Trp Lys Leu
1 5 10 15

Ser Phe Ser Ile Val Ser Leu Cys Asn His Leu Thr Arg
20 25

<210> 9

<211> 29
<212> PRT
<213> Human

<400> 9

Thr Cys Asp Cys Leu Lys Lys Leu Asn Ala Arg Arg Lys Leu Lys Gly
1 5 10 15

Ala Ile Leu Thr Thr Met Leu Ala Thr Arg Asn Phe Ser
20 25

<210> 10

<211> 28

<212> PRT

<213> Human

<400> 10

Val Ser Glu Gln Ile Lys Lys Asn Phe Ala Lys Ser Lys Trp Lys Gln
1 5 10 15

Ala Phe Asn Ala Thr Ala Val Val Arg His Met Arg
20 25

<210> 11

<211> 32

<212> PRT

<213> Human

<400> 11

Met Asp Thr Ala Gln Lys Lys Leu Gln Glu Phe Asn Ala Arg Arg Lys
1 5 10 15

Leu Lys Ala Ala Val Lys Ala Val Val Ala Ser Ser Arg Leu Gly Ser
20 25 30

<210> 12
<211> 28
<212> PRT
<213> Human

<400> 12

Gly Glu Asp Ser Gly Arg Lys Pro Glu Arg Arg Leu Lys Thr Thr

1

5

10

15

Arg Leu Lys Glu Tyr Thr Ile Lys Ser His Ser Ser
20 25

<210> 13
<211> 20
<212> DNA
<213> Human

<400> 13
ggccggatga ggacacctgagg

20

<210> 14
<211> 21
<212> DNA
<213> Human

<400> 14
tccacatcccc accccagact c

21

United States Patent & Trademark Office
Office of Initial Patent Examination -- Scanning Division



SCANNED # 2

Application deficiencies found during scanning:

Page(s) _____ of _____ were not present
for scanning. (Document title)

Page(s) _____ of _____ were not present
for scanning. (Document title)

Scanned copy is best available.

Drawings